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# A conjugate vaccine composed of a heat shock protein 60 T-cell epitope peptide (p458) and *Neisseria meningitidis* type B capsular polysaccharide☆

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**Abstract**

*Neisseria meningitidis* type B is a major world-health problem. The Meningococcus type B capsular polysaccharide (MnB) is very poorly immunogenic and no vaccine to the antigen exists. Here, we conjugated the MnB to a T-cell carrier peptide (p458) derived from the self-60 kDa heat shock protein molecule. The conjugate vaccine was effective in inducing long-lasting IgG antibodies to the MnB antigen in mice. The vaccine was also immunogenic when injected in PBS. Thus, the p458 carrier peptide can induce T-cell help for the switch to IgG Ab to the MnB antigen.


*Keywords:* Meningococcus B; Peptide; Heat shock protein 60; Vaccination

## 1. Introduction

*Neisseria meningitidis* (Meningococcus) type B is responsible for many of cases of meningitis throughout the world, particularly in children [1]. The Meningococcal type B capsular polysaccharide (MnB) is a T-independent (TI) antigen. TI antigens are not recognized by helper T-cells and so activate only B-cells leading predominantly to a short-lived IgM response and to almost no immunological memory [2]. Protein carriers have been conjugated to TI antigens to recruit T-cell help to induce B-cells to switch to IgG secretion and to generate immunological memory [3]. Indeed, an immunogenic carrier can induce high titers of IgG antibodies (Abs) to a conjugated capsular polysaccharide (PS), for example, that of the Mn type C (MnC) [4]. New conjugated vaccines to MnC using tetanus toxoid (TT) and diphtheria toxoid as carriers are now available [5], and have been effective in inhibiting Meningococcus type C meningitis in populations at risk [6].

Both MnB and MnC are homopolymers of sialic acid with a difference in their glycosidic linkage: α(2–8) sialic acid in MnB compared to α(2–9) sialic acid in MnC [7]. This seemingly minor difference leads to a major difference in the immunogenicity of these antigens; MnB is a very poor immunogen while MnC is an immunogenic TI antigen. MnB has structural homology with a self-antigen,

*Abbreviations:* Ab, antibody; DDW, double distilled water; HSP60, heat shock protein 60; MnB, meningococcus type B PS; MnC, meningococcus type C PS; OMV, outer membrane vesicle protein; p458, a self-HSP60 T-cell epitope; PS, capsular polysaccharide; PSA-NCAM, polysialylated adhesion molecule NCAM; TI, T-independent; TT, tetanus toxoid

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the polysialylated adhesion molecule NCAM (PSA-NCAM) that is expressed in mammalian fetal tissues [8]. In addition, cross-reactivity between anti-MnB Abs and brain extracts has been reported [9]. Apparently, the structural homology of MnB with mammalian tissues leads to its very poor immunogenicity [10]. Thus, there is currently no effective vaccine against Meningococcus type B based on the PS. Vaccines to Meningococcus type B, based on the outer membrane vesicle protein (OMV) have been used [11] and others are undergoing clinical trials [12–14]. Nevertheless, a conjugated MnB vaccine would be useful for the induction of protection against all the bacterial serotypes that share this PS.

Here, we studied vaccination to Meningococcus type B using a conjugate vaccine. Our model is based on the p458 peptide carrier, a T-cell epitope that originates from the self-heat shock protein 60 (HSP60) molecule. HSP60 is a strong immunogen. Self and foreign HSP60 are up-regulated at sites of inflammation, are ligands for innate immune receptors [15,16] and serve as antigens for T-cells [17]. Peptide p458 was found to be a T-cell epitope, and was previously used to develop conjugate vaccines to *Streptococcus pneumoniae*, *Salmonella typhi* (then named CP1) and to a murine cytomegalovirus CTL-epitope [18–21]. We employed the p458 carrier in a conjugate vaccine with MnB (MnB-p458) and tested the ability of this vaccine to induce long-lasting serum IgG Abs to the PS antigen.

## 2. Materials and methods

### 2.1. Mice

Female BALB/c mice were obtained from Harlan Olac (Bicester, UK) and were used at the age of 8 weeks unless indicated otherwise. Experiments were done according to the guidelines and under the supervision of the Animal Welfare Committee.

### 2.2. Peptide p458

Peptide p458 was synthesized using an automated multiple peptide synthesizer (Abimed model AMS 422; Langenfeld, Germany), according to the company's instructions for *N*-α-fluorenylmethoxycarbonyl (Fmoc) synthesis. The purity of the peptide was tested by analytical reversed-phase HPLC and amino acid analysis. Peptide p458 originates from murine HSP60 at positions 458–474; its sequence is NEDQKIGIEIIKRALKI.

### 2.3. Mn band MnC

MnB and MnC were prepared as previously described [22,23]. The Meninggococcus types B or C were cultivated in modified Frantz medium [22] in 20 L batches until stationary growth was reached. Bacteria were removed by centrifugation at 3000 × *g* for 10 min. The supernatant was collected and 1% hexadecyl-trimethylammonium bromide (Panreac Quimica SA; Barcelona, Spain) was added and centrifuged (10,000 × *g*, 25 min). PS extraction was as follows: the pellet was dissolved in 1 L of DDW followed by the addition of 1 L of 2 M $CaCl_2$ solution. Ethanol at 25% was then added and the mixture was incubated for 1 h at −20 °C and then was centrifuged (20,000 × *g*, 20 min). The supernatant was added slowly but with agitation to an icy 80% ethanol, incubated for 1 h at 20 °C and then centrifuged (3000 × *g*, 10 min). The semi-purified PS was then dissolved in NaAc 0.1 M saturated at pH 7 and vigorously mixed equally (v/v) with a phenol solution (100 g/40 mL of NaAc 0.1 M saturated, pH 7). The mixture was centrifuged (35,000 × *g*, 15 min) and the upper phase was collected. The phenol-extraction step was repeated and the resulting solution was dialyzed for 24 h against $CaCl_2$ (0.1 M). The dialyzed solution of PS was ultracentrifuged at 100,000 × *g* for 3 h and precipitated with ethanol to 80%, followed by centrifugation at 3000 × *g* for 10 min.

### 2.4. MnB or MnC conjugation

Conjugates were made using a single batch of MnC or MnB. The PS molecules were dissolved in double distilled water (DDW) to a final concentration of 5 mg/mL and were activated (stirred continuously) with 0.1 mL of cyanogen bromide (20 mg/mL in acetone) in the presence of 30 mM triethylamine (Aldrich; Milwaukee, WI, USA) in acetone at pH 7. The spacer 6-aminohexanoic acid (BDH Chemicals; Poole, England; 10 mg/mL in DDW) was added 2 min later to the activated PS molecules and then incubated for 2 h at 4 °C. We then added 12 mg of water-soluble diimide: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (CDI) (Aldrich) and 7 mg of p458 peptide to the solution. The pH was adjusted to 6 at room temperature. Four hours later, 12 mg of CDI was again added, and the mixture was incubated overnight, and then dialyzed at 4 °C against DDW. The sialic acid content was measured by the thiobarbituric acid assay [24]. Amino acid analysis (Chemical Services, Weizmann Institute) was used to determine the amount of peptide coupled to the PS molecules. The PS to peptide ratios by weight of the different batches were 1.5–0.7 for MnB-p458 and 2–0.9 for MnC-p458.

### 2.5. Immunization

Mice were injected s.c. on the back three times at 2-week intervals with the indicated dose of MnB or MnC in 0.1 mL, either conjugated or unconjugated. Unless indicated otherwise, the vaccines were emulsified in one volume of IFA per one volume of PBS containing the antigen. In the indicated experiments, the vaccines were administered in PBS alone. The dose of unconjugated p458 peptide mixed with PS (not conjugated) was in accordance with its relative amount in the appropriate conjugate. The administration of MnB and MnC

conjugate together (MnB-p458 + MnC-p458) was done with the indicated doses of PS in 0.1 mL. The mice were bled 4 weeks after the last boost or as indicated. The blood samples were centrifuged at 14,000 × *g* for 10 min, and the sera were collected and stored at −20 °C.

### 2.6. ELISA assay

Maxisorb 96-well plates (Nunc; Roskilde, Denmark) were coated overnight at 4 °C with 10 μg/mL of MnC or MnB in PBS. Between the different stages, the plates were washed three times with 0.02% Tween-20 in PBS (TPBS). Each plate was blocked with 2% skim milk (DIFCO, Detroit, MI, USA) in PBS. Serum samples were diluted 1–50 in 0.2% skim milk in PBS, and incubated in the well for 2 h at 37 °C. The detection of IgG Ab was done using goat anti-mouse IgG coupled to alkaline phosphatase (Jackson; West Grove, PA, USA) diluted 1:1000, and incubated for 45 min at 37 °C. IgM was detected using goat anti-mouse IgM coupled to alkaline phosphatase (Jackson) diluted 1:1000. IgG subtypes were detected using goat anti-mouse IgG subtypes (IgG1/2a/2b/3) coupled to alkaline phosphatase (Southern Biotechnology Association, Ink; Birmingham, AL, USA) diluted 1:1000. The substrate solution containing 0.6 mg/mL of *p*-nitrophenylphosphate (Sigma; Rehovot, Israel) in diethanolamine–$H_2O$, pH 9.8, was then added. A strong color was detectable usually after 15 min to 1 h of incubation. The OD was read at wavelength 405 nm ($OD_{405\,nm}$).

### 2.7. Statistics

Results were analyzed using Student's *T* test, the Alternate Welch *T* test or the Nonparametric (Wilcoxon or Mann–Whitney) test computed using software package InState 2.01 for the Macintosh computer.

## 3. Results

### 3.1. Vaccination with low doses of the conjugate vaccine induces IgG Abs to MnB

Based on an earlier study in which a p458-conjugated Pneumococcal vaccine was effective at 2 μg PS per mouse (30), we vaccinated mice three times with 0.2, 2 or 20 μg PS with the conjugate (MnB-p458), the PS alone (MnB) or an unconjugated mixture of PS and p458 (MnB + p458) emulsified in IFA. Sera were collected 2 weeks after the third injection and the anti-MnB IgG Ab levels were measured by ELISA. Fig. 1A shows that vaccination with the MnB-p458 conjugate at 0.2 or 2 μg per mouse was somewhat more effective than vaccination with 20 μg in the induction of IgG Abs to MnB; the effectiveness of only 0.2 μg per mouse did not differ from that of 2 μg. Further experiments were performed using the 2 μg dose per mouse.

The MnC molecule is effective in conjugate vaccines, and we compared conjugates of the MnB and MnC molecules. We tested two doses of vaccine: 2 and 20 μg PS per mouse of the MnC-p458 conjugate or the MnC + p458 mixture, emulsified in IFA. The sera were collected and tested as described for MnB (Fig. 1B). The injection of 20 μg of MnC-p458 induced a significantly higher anti-MnC IgG Ab response than did the 2 μg dose. Hence, MnB and MnC conjugates manifest a different dose–response profile; the MnB conjugate is more effective at lower doses than is the MnC conjugate.

### 3.2. Requirement for three immunizations

To determine the number of immunizations required to induce IgG Abs, we injected mice three times with the MnB-p458 conjugate, or with MnB + p458 or MnB emulsified in IFA.

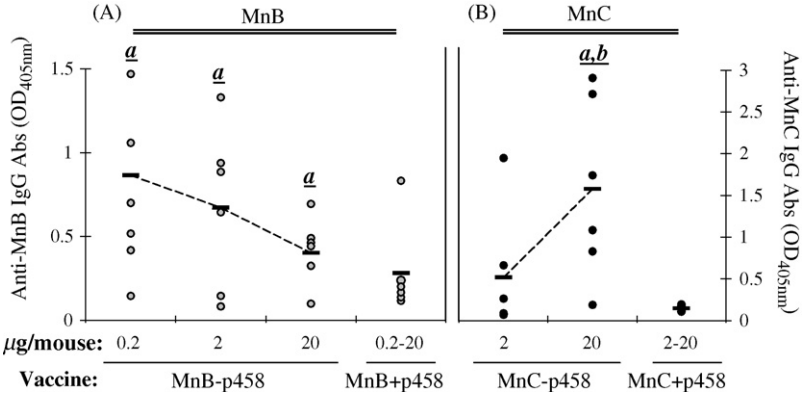


Fig. 1. Vaccine doses. Groups of six mice were vaccinated three times at 2-week intervals with 0.2, 2 or 20 μg per mouse of MnB-p458, MnB + p458* (A) or with either 2 or 20 μg per mouse of MnC-p458 or MnC + p458* (B), and bled 1 month later. The sera of individual mice were assayed by ELISA for anti-MnB or anti-MnC IgG Abs. Each circle represents a single mouse, the horizontal line represents the mean OD and the doted line represents the change, in this and in subsequent figures. *Note that since there was little Ab production induced by unconjugated MnB + p458 or by unconjugated MnC + p458 in the doses of 0.2, 2 or 20 μg, we show the Ab responses to the various doses collectively. [a] $p < 0.046$ compared to MnB + p458, 0.2–20 μg/mouse. [b] $p < 0.064$ compared to MnC-p458, 2 μg/mouse.

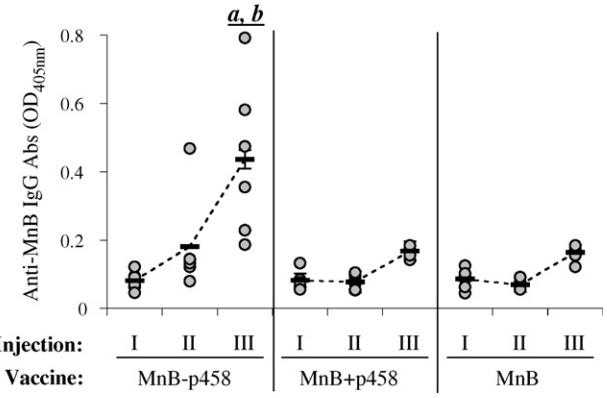


Fig. 2. Three injections of the MnB-p458 vaccine are needed to induce anti-MnB IgG. Groups of six mice were vaccinated three times with 2 μg per mouse of MnB-p458, MnB + p458 or MnB and bled 2 weeks after the first (I), second (II) and third (III) vaccinations. The sera of individual mice were assayed by ELISA for IgG Ab to MnB. [a] $p < 0.049$ compared to MnB-p458, I and II. [b] $p < 0.034$ compared to MnB + p458, III and MnB III.

The mice were bled 2 weeks after each vaccination. Fig. 2 shows that the mice produced IgG Abs to MnB after three immunizations with the MnB-p458 conjugate. The other vaccines failed to induce significant amounts of IgG Abs.

### 3.3. MnB-p458 induces mainly IgG1 Abs

Fig. 3 shows that most of the IgG Abs induced in response to MnB-p458 were of the IgG1 isotype. Low levels of the IgG2a, IgG2b and IgG3 isotypes were observed. Anti-MnB IgM and lower levels of IgG3 were induced both by the conjugate and by unconjugated MnB. These IgM and IgG3 Abs declined spontaneously and could not be detected after 2 months (data not shown).

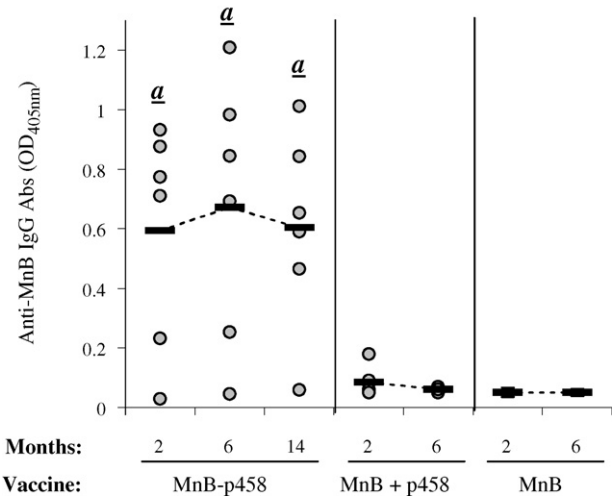


Fig. 4. Long-lasting Abs to MnB. Groups of six mice were injected three times with MnB-p458, MnB + p458 or with MnB alone. The mice were bled at the age of 6 months (2 months after immunization), at the age of 10 months (6 months after immunization) and at the age of 18 months (14 months after immunization). The sera of individual mice were tested by ELISA for Ab to MnB. [a] $p < 0.008$ compared to MnB + p458 or to MnB.

### 3.4. MnB-p458 induces long-lasting Ab to MnB

Vaccines to Meningococcus type B ideally should be effective from vaccination at childhood and into adulthood [25]. We examined the duration of IgG Abs to MnB in the sera of mice injected with the conjugate MnB-p458 or with MnB alone or mixed with the p458 peptide, emulsified in IFA. The mice were bled at 2, 6 and 14 months after the last boost (Fig. 4). Fourteen months after the last boost, at the age of about 18 months, the amount of IgG Abs was unchanged. Thus, long-lasting IgG Ab was induced by the

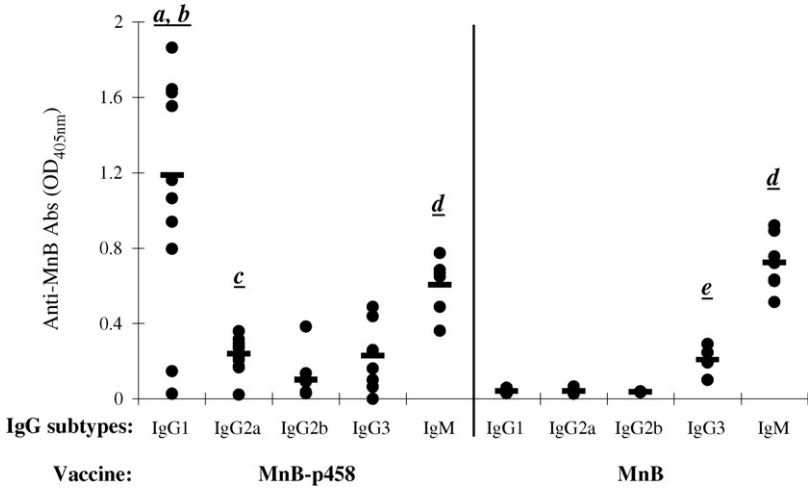


Fig. 3. MnB-p458 induces anti-MnB IgG1 Ab. Mice were vaccinated three times with MnB-p458 (10 mice) or MnB (5 mice) and bled 1 month following the last immunization. The sera of individual mice were assayed by ELISA for the presence of anti-MnB IgG1, IgG2a, IgG2b, IgG3 or IgM Abs. Note that mice vaccinated with MnB-p458 that did not produce IgG1 Abs, also lacked other IgG subtypes. [a] $p < 0.042$ compared to other MnB-p458 subtypes. [b] $p < 0.0005$ compared to MnB IgG1. [c] $p < 0.0002$ compared to MnB IgG2a. [d] $p < 0.002$ compared to MnB-p458 IgG2a, IgG2b and IgG3 and MnB IgG2a, IgG2b and IgG3. [e] $p < 0.028$ compared to MnB IgG1, IgG2a and IgG2b.

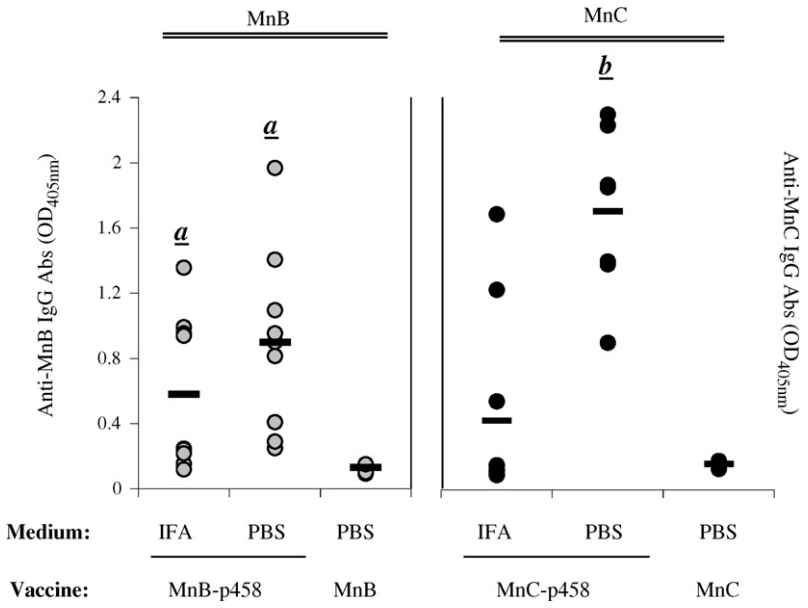


Fig. 5. PBS is an effective vehicle to induce anti-MnB and anti-MnC IgG Abs. Groups of eight to nine mice were immunized three times with 2 μg of MnB-p458 or MnC-p458 in IFA or in PBS or with MnB or MnC in PBS. The mice were bled 1 month following the last immunization and the sera were tested individually by ELISA for anti-MnB Abs. [a] $p < 0.022$ compared to MnB in PBS. [b] $p < 0.0003$ compared to the other MnC groups.

MnB-p458 conjugate vaccine. Note, however, that not all the mice produced detectable amounts of Abs. None of the mice immunized with MnB + p458 or with MnB injected alone produced IgG Abs to MnB. In conclusion, IgG serum Abs to MnB persist in responding mice.

### 3.5. Adjuvant is unnecessary

The adjuvant is a critical factor in vaccine design. We tested whether the p458 carrier can induce IgG Ab production without an added adjuvant; vaccination with MnB-p458 in IFA or in PBS was compared (Fig. 5, left panel). The MnB-p458 conjugate appeared to be more effective when injected in PBS than in IFA; however, the differences were not statistically significant at this number of mice. In any case, we can conclude that IFA is not essential to the immunogenicity of MnB-p458.

We also vaccinated mice with the suboptimal dose of 2 μg of MnC-p458 in PBS, or emulsified in IFA. A suboptimal dose can highlight differences in the effectiveness of vaccines that may seem to be equal at optimized doses. Surprisingly, Fig. 5 (right panel), shows that mice immunized with MnC-p458 in PBS produced higher amounts of Abs to MnC. Moreover, all of the vaccinated mice produced Abs. In contrast, most of the mice injected with only 2 μg of MnC-p458 in IFA failed to produce detectable Abs ($p = 0.0003$; see also Fig. 1). Thus, the p458 carrier seems to eliminate the need for an external adjuvant; the conjugate in PBS is sufficient for inducing an IgG immune response to the attached PS. A vaccine that is effective when injected with no adjuvant is advantageous in human vaccination.

### 3.6. Specificity of conjugated vaccine; co-administration of conjugated MnB and MnC induces Abs to both PSs

It has been reported that the administration of conjugated MnC could induce some cross-reactive Abs to both MnC and MnB [26]. To investigate possible cross-reactivity between

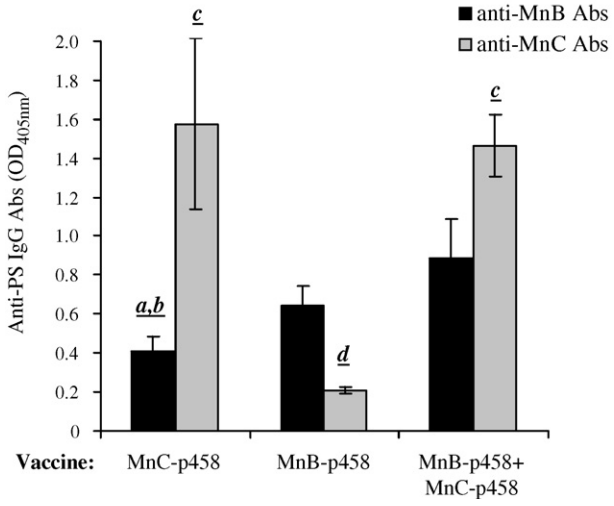


Fig. 6. Reciprocal induction of cross-reactive Abs to MnB or MnC. Mice were injected three times with MnB-p458, MnC-p458 or with MnB-p458 + MnC-p458. The mice were bled 4 weeks after the last boost. The sera of individual mice were tested by ELISA for Ab to MnB or MnC. The mean and standard error were determined. [a] $p < 0.0687$ compared to MnB-p458, anti-MnB. [b] $p < 0.0469$ compared to MnB-p458 + MnC-p458, anti-MnB. [c] $p < 0.0266$ compared to MnB-p458, anti-MnC. [d] $p < 0.0409$ compared to MnC-p458, anti-MnB.

anti-MnB and anti-MnC Abs, we immunized mice with MnB-p458 or MnC-p458 and assayed IgG Abs binding to either MnC or MnB. Fig. 6 shows that each vaccine induced Abs that bound to the specific antigen with a three- or four-fold higher OD than to the other PS. Thus, both conjugated vaccines were relatively, but not absolutely specific. We also tested whether MnB-p458 and MnC-p458 administered together might be effective in inducing IgG Abs to each PS. The presence of MnC-p458 did not inhibit the induction of IgG Abs to MnB. Thus, there is no interference between the two conjugated vaccines.

## 4. Discussion

The MnB antigen is a poor immunogen and the induction of IgG Abs to conjugated MnB is challenging. Nevertheless, in our experiments, MnB conjugated to p458 functioned as an immunogenic vaccine. We were able to induce long-lasting IgG Abs to MnB in most of the vaccinated mice after three immunizations with the MnB-p458 conjugate. Most of the Ab was of the IgG1 isotype, which marks the secondary Ab response, the IgG switch and memory [3]. Interestingly, the amount of Ab in the sera of mice injected with only 2 or 0.2 μg of the MnB-p458 conjugate was somewhat higher than that of mice injected with 20 μg of the conjugate. Moreover, no adjuvant was needed to induce an Ab response to MnB. Abs induced to MnB or MnC were relatively specific. Finally, the MnB-p458 conjugate administered together with the MnC-p458 conjugate induced Abs to both MnB and MnC; there was no interference.

In comparison to MnB, the MnC of Meningococcus type C is an immunogenic TI antigen that has been used as a vaccine [27] conjugated to diphtheria toxoid or to TT [28]. Here, we conjugated MnC to the p458 peptide (MnC-p458) to create a comparable vaccine to the less immunogenic MnB antigen. The injection of 20 μg of the MnC-p458 conjugate was more effective than 2 μg. Yet, at 2 μg per mouse, MnC-p458 in PBS was significantly more immunogenic than MnC-p458 in IFA. Hence, conjugated p458 can induce an immune response to both PSs and may be used as its own adjuvant. Nevertheless, the dose–response profile of the conjugate seems to be determined by the particular PS rather than by the p458 carrier.

There is a clear correlation between Abs to MnC and resistance to Meningococcus type C infection [29]. Less research has been done regarding the role of Abs to MnB in preventing Meningococcus type B infection. Nevertheless, Abs to MnB were shown to be protective in passive transfer of sera [7] and could activate bactericidal mechanisms [30]. Despite the difficulty in inducing anti-MnB Abs, such Abs are likely to recognize all Meningococcus type B strains because they all share the MnB antigen.

Why is MnB such a poor immunogen? MnB has three characteristics that can combine to decrease its immunogenicity: (i) MnB is a TI antigen, so there is no T-cell help; (ii) MnB is identical to a self-carbohydrate widely expressed in mammals, mainly during development [31]; (iii) the unique molecular conformation of MnB [32] may allow it to escape B-cell recognition due to rapid elimination [33]. Indeed, individuals usually fail to produce anti-MnB IgG Abs even after infection or carriage of Meningococcus type B bacteria [34]. Moreover, anti-MnB Abs have lower avidity in comparison to anti-MnC Abs [35]; this is probably due to the preponderance of Ab of the IgM isotype in the serum [33]. There is no evidence for suppressor T-cells in the response to MnB. Moreno et al. reported that athymic mice or mice that were treated with cyclophosphamide were also poor responders to this unique molecule [36].

Conjugated vaccines composed of MnB attached to TT or CRM197 have had limited success in inducing IgG Abs to MnB [8,37]. Similar to our results, Abs were mostly raised after the third injection, but, unlike the Abs reported here, those Abs were short-lived [8]. Another approach, in which a chemically modified MnB was conjugated to Meningococcal B Porin, was able to elicit bactericidal Abs to the PS moiety in mice and monkeys [38]. Native MnB was not effective in inhibiting the bactericidal effect; apparently, the modified MnB mimics an MnB conformation expressed on the bacterium [39]. Thus, the immunogenicity of MnB can be increased by conjugation and modification. The observation that the MnB molecule was more effective when administered at lower doses could be attributed to the immunological paralysis associated with TI antigens [40,41].

Vaccination in PBS was effective for both MnB-p458 and MnC-p458 conjugates. Adjuvants are thought to promote an innate immune response that results in enhancing the Ab response [42]. Vaccination without adjuvant could significantly reduce adverse reactions [43]. In addition, the mice we vaccinated at the age 8 weeks with the MnB conjugated vaccine developed long-lasting serum Ab. It is conceivable that repeated exposure to either endogenous [44] or bacterial HSP60 [45], might help maintain immune memory to the PS antigens conjugated to the self-HSP60 peptide, p458. The mechanism by which the p458 conjugates vaccinate when administered in PBS without adjuvant remains to be determined; it is known that the parent HSP60 molecule can activate Toll-like receptors [16], and perhaps the p458 conjugate can do likewise.

A Meningococcus type C vaccine trial in England was associated with reduction in Meningococcus type C infections but also with an increase in Meningococcus type B infections in the vaccinated population [46]. Increased episodes of infection or carriage involving other bacterial strains after a specific vaccination are well-documented [47–49]. Thus, the administration of combined vaccines against several bacterial strains may be advantageous. In our hands and others [50], the Ab response to MnB PS was slightly enhanced if MnC was present in the conjugate vaccine. The combination of the Meningococcus PSs might form a vaccine against both Meningococcus types B and C.

The approved carriers for most conjugated vaccines for a variety of bacteria have been limited, and diversifying the

pool of approved carriers might soon be needed [51,52]. A possible solution might be the use of T-cell epitope peptides rather than whole proteins. Peptides are easy to manufacture and might be safer to use than a whole protein since they are more predictable in their immune behavior. More importantly, peptides have been shown to be as immunogenic as whole proteins [53]. In our previous paper, both TT and its T-cell epitope peptide p30 showed similar effectiveness in inducing Ab and protection to *Pneumococcus* type 4 challenge when conjugated to the specific type 4 PS. Moreover, we have found that *Pneumococcus* type 4 PS conjugated to p458 was a more effective vaccine than the PS conjugated to whole HSP60 [18]. Nevertheless, the MHC restrictions of peptides such as p458 might impose limitations in the use of peptide carriers. Indeed, carrier p458, but not carrier p30, was shown to be restricted to some MHC alleles [18]. A chemically modified HSP60 peptide, or a combination of several peptides might elicit responses among more diverse MHC alleles.

The question of autoimmunity to an HSP60 peptide should be considered. Vaccination with conjugated vaccines to p458, however, has never been observed to induce an autoimmune disease [19]. Moreover, we previously showed that there is no production of Abs to p458 when injected in conjugation to Pneumococcus type 4 PS [18], or to MnB (data not shown). Vaccination with HSP60 protein, HSP60 DNA or HSP60 peptides has been found to prevent or arrest autoimmune inflammation both in type 1 diabetes and in arthritis [54–58]. Healthy humans manifest T-cell reactivity to HSP60 [59]; T-cells reactive to HSP60 are present from birth [60,61]; anti-HSP60 T-cells accumulate at sites of inflammation [62]. In fact, repeated natural immune exposure to HSP60 might contribute to the persistence of IgG Abs following vaccination with p458 conjugates. Up-regulation of HSP60 at the site of an infection might even mobilize anti-HSP60 T-cell help in the immune response in suitably vaccinated hosts. At this point, there is no evidence indicating that p458 conjugates induce autoimmune disease.

The question of autoimmunity to MnB is also moot. More study is needed to assess anti-MnB Ab binding to antigens expressed in the brain and during fetal development. Pregnant rabbits that were vaccinated with MnB passively transferred anti-MnB Abs to their offspring and rendered them resistant to *Escherichia coli* K1; K1 has an identical PS structure to MnB [63]. No pregnancy failure was reported [64]. Similarly, experiments in animal models [8,65] and humans [66,67] showed no autoimmune disease. Nevertheless, more study is needed to verify the safety of any MnB vaccine.

A vaccine against the MnB antigen might be useful in diseases other than MnB infection. In multiple sclerosis, for example, there is evidence that re-expression of the MnB homolog, PSA-NCAM, on the axonal surface can prevent myelin-forming cells from attaching to the axon and inhibit remyelination [68]. Ab to MnB and its PSA-NCAM homolog might help prevent the arrest of remyelination in multiple sclerosis. In addition, PSA-NCAM is expressed by different tumors [69–71], and probably serves as a de-adhesion molecule in tumor metastasis [72]. Here, too, Ab to MnB might inhibit the metastasis of some tumors. Another highly virulent pathogen is the *E. coli* K1, which shares the MnB structure, expressing a PSA-NCAM-like molecule. The construction of a vaccine aimed at Meningococcal type B PS can perhaps induce resistance to this bacterium as well.

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