Activation of TRIF-dependent and independent immune responses by neisserial heat shock protein complex vaccines

A commentary on:
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ABSTRACT

Heat shock protein Complex (HspC) vaccines are composed of Hsp purified from pathogenic bacteria along with their chaperoned protein cargo. Mouse studies have shown that HspC vaccines can induce a strong immune response against pathogenic bacteria without addition of an exogenous adjuvant. These vaccines are now entering clinical trials. It was predicted, but not previously tested, that HspC vaccines induce an immune response due to the activation of Toll-Like Receptors (TLR) by their component Hsp. Recently we tested this supposition and found that while this held true for the cellular response to neisserial HspC vaccines, strong antigen-specific antibody responses were surprisingly generated in mice deficient in MyD88 and thus most TLR signaling. This suggested an unidentified mechanism by which HspC vaccines induce an antibody response. We have now examined the antigenic profile of this response and found no evidence that this is due to the induction of T-independent antibodies. Examination of the MyD88-dependent signaling pathways involved in the cellular response to neisserial HspC showed that both TRIF-dependent and TRIF-independent pathways are activated, each resulting in the secretion of different cytokines. Hence the mechanism of action of HspC vaccines is clearly more complicated than originally thought.

Keywords:

Heat shock protein complex, vaccine, *Neisseria lactamica*, *Neisseria meningitidis*, TRIF, Toll-like receptors, macrophages

INTRODUCTION

Heat shock proteins (Hsp) are highly conserved molecules possessing a range of properties, including the abilities to act as protein chaperones and to trigger innate immune responses via the activation of Toll-Like Receptors (TLR).[1, 2] The combination of these properties has led to the development and testing of a novel technology, involving the use of heat shock protein complexes (HspC) as vaccines.[3]

HspC vaccines comprise Hsp from pathogenic bacteria, which are purified along with their chaperoned bacterial protein cargo. Mouse studies have shown that HspC vaccines, when delivered either by injection or via a mucosal route, can induce a strong immune response against pathogenic bacteria from which the HspC are purified, and importantly without the addition of an exogenous adjuvant.[4-6] The chaperoned bacterial cargo provides the vaccine antigen, whilst the intrinsic ability of the component Hsp to activate innate immune receptors is believed to provide the vaccines' adjuvant activity.

While it was strongly believed that HspC vaccines trigger an immune response via the activation of cell surface TLR, and in particular TLR2 and TLR4, there was no empirical evidence to support this supposition. In a study recently published in *Vaccine* we reported the first evaluation of the mechanism of action of an HspC vaccine.[6] This showed that, as predicted, the cellular response of mice to neisserial HspC vaccines was completely dependent on the TLR adaptor protein MyD88. Surprisingly however, we found that the antibody response to this vaccine was MyD88-independent, suggesting that the humoral-response to this vaccine was induced by a mechanism that did not involve TLR activation.

HSPC VACCINE INDUCED CELLULAR RESPONSE

In this recent study we found that vaccination of wildtype mice with neisserial HspC induced a Th1-dominant cellular response, but that this response was completely absent in MyD88 deficient mice.[6] As TLR2 and TLR4 are both dependent on MyD88 this is consistent with the original premise that HspC induce immunity via activation of cell surface TLR. However there remain several unanswered questions.

First, it is not yet understood why neisserial HspC vaccination of mice induced a strong Th1-type response (including vaccine-induced memory), yet no Th17-type response was detectable. HspC vaccines prepared from other bacteria are potent inducers of Th1-type responses in mice,[4] as are Hsp,[7, 8] so the fact we observe a Th1

profile with neisserial HspC is not surprising. The ability of Hsp to induce Th17-type responses, though less well studied, has also been reported.[9] We have not yet determined why a Th17 response was not detectable following neisserial HspC vaccination or direct stimulation of mouse splenocytes.[6] However it is perhaps worth noting that we have previously detected an increased Th17 response at the site of infection in mice prophylactically vaccinated with HspC against *Helicobacter pylori*,[5] which suggests the ability of HspC to induce a Th17 response might either vary between the bacterial source of the formulation, or require a live *in vivo* challenge (something we have not performed for the *Neisseria* studies).

Second, it is not yet known precisely which TLRs are activated by the HspC. In our recent study we were able to demonstrate that neisserial HspC activated multiple TLRs including TLR2, but the response to this vaccine was not completely dependent on either TLR2 or TLR4. We have since further dissected the pathways activated by neisserial HspC. The signaling pathways downstream of MyD88 that follow TLR2 and TLR4 activation can largely be differentiated by analysis of another TLR adapter protein called TRIF (TIR-domain-containing adapter-inducing interferon-β). TLR4 activation induces signaling down two pathways, one of which involves TRIF, while TLR2 activation is largely believed to be TRIF-independent (although there is some evidence of TRIF involvement in TLR2 signaling).[10] While TRIF is also essential for signaling via intracellular TLR3, it is not believed to be involved with any other TLR.

We therefore generated TRIF-deficient RAW264.7 macrophages (supplemental Figure S1) and measured their cytokine response to stimulation with *N. lactamica* HspC, revealing an intriguing extra level of complexity. While the secretion of some cytokines in response to *N. lactamica* HspC was unaffected by TRIF deficiency (for example TNFα), others such as IL-6 were shown to be TRIF-dependent (Figure 1). In addition to supporting our previous observation that neisserial HspC activates multiple TLR, these findings also indicate that activation of individual TLRs by HspC can result in differential cytokine secretion.

As mentioned above, *N. lactamica* HspC induces a Th1-type memory response.[6] We thus evaluated IL-12 secretion (as a key driver of Th1-immunity) in the RAW264.7 cells we generated for this current study. Unfortunately we found that RAW264.7 cells do not secrete IL-12 when activated via TLR4, as previously reported.[11]

ANTIBODY RESPONSE

An unexpected finding from our evaluation of their mechanism of action was that immunization of mice with both *N. lactamica* and *N. meningitidis* HspC induced antigen-specific antibody responses, even in MyD88 deficient mice.[6] If anything, the response to these vaccines was greater in mice lacking the ability to signal via all the MyD88-dependent TLR than in wildtype mice. This was surprising, given our initial premise that HspC vaccines adjuvant an immune response via TLR activation.

Our study did not identify the mechanism by which these HspC vaccines induced a strong antibody response, and this remains to be determined. However, we did examine the possibility that the HspC were inducing a T-cell independent antibody response. This possibility was raised by studies on T-independent antibody responses induced in B-cells activated with peptide liposomes that have been shown to be mediated via pathways involving TRIF and not MyD88.[12]

T-independent antigens typically either induce an antibody response via the activation of TLR (excluded above) or are specific types of antigen, such as polysaccharides, against which an antibody response is generated without T cell help.[13] We explored the latter possibility, but while minor differences were apparent in the antigens detected by HspC-specific antibodies generated by HspC vaccination of wildtype and *Myd88*$^{-/-}$ mice (Figure 2), the profiles of the western blots were overall very similar. As no antibodies against specific types of antigens were evident in the *Myd88*$^{-/-}$ mice, the ability of neisserial HspC to induce antibodies in the absence of MyD88 does not appear to be the result of a T-independent humoral response.

More work is therefore required to identify how this occurs. It is possible that it is via a TRIF-dependent, MyD88-independent pathway as has been suggested for peptide liposomes.[12] It should also be remembered that MyD88 deficient mice still have functional TLR3 although it seems unlikely, though not impossible, that the vaccine would act via this intracellular innate receptor.

SUMMARY

In summary, HspC vaccines are providing a potentially valuable new vaccine technology that allows the generation of a cellular and antibody response to pathogenic bacterial antigens without the complication of needing to add an exogenous adjuvant. This has clear advantages with respect to safety, manufacturing

and progression through clinical trials. Indeed, this technology has recently completed its first clinical trial in a pneumococcal vaccine clinical trial.

The mechanism of action of these vaccines is clearly more complicated than originally thought, particularly with respect to how antibody responses are generated. How these vaccines can induce a strong antibody response, without an exogenous adjuvant, and without activation of core TLRs remains a fascinating question.

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CONFLICT OF INTEREST STATEMENT

CAC is an employee of ImmunoBiology Limited, a company developing vaccines targeted to dendritic cells using Heat shock proteins. The work was partially funded by Immunobiology Limited.

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FIGURE LEGENDS

FIGURE 1

TRIF-dependency of the macrophage response to neisserial HspC

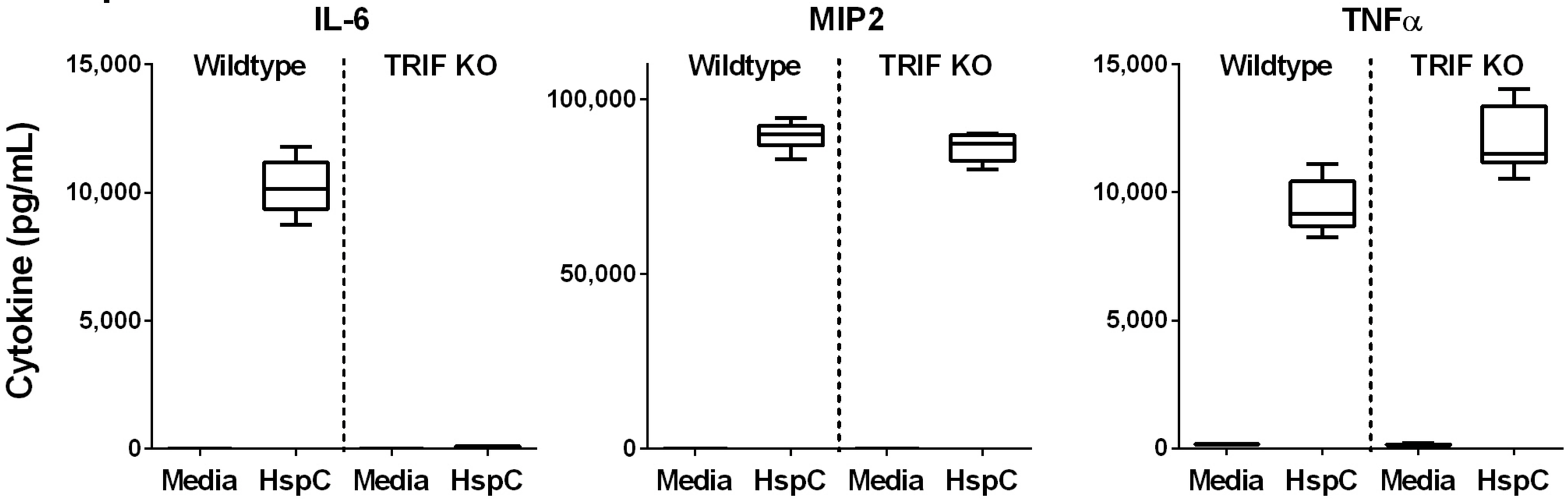
TRIF-deficient RAW264.7 mouse macrophages (TRIF KO) were made as described in supplemental information. *N. lactamica* HspC was prepared as described.[6] Cell culture experiments and enzyme linked immunosorbent assay (ELISA) were performed as described previously for splenocytes,[6] except RAW264.7 macrophages were serum starved overnight prior to stimulation. The experiment was performed twice (both shown).

FIGURE 2

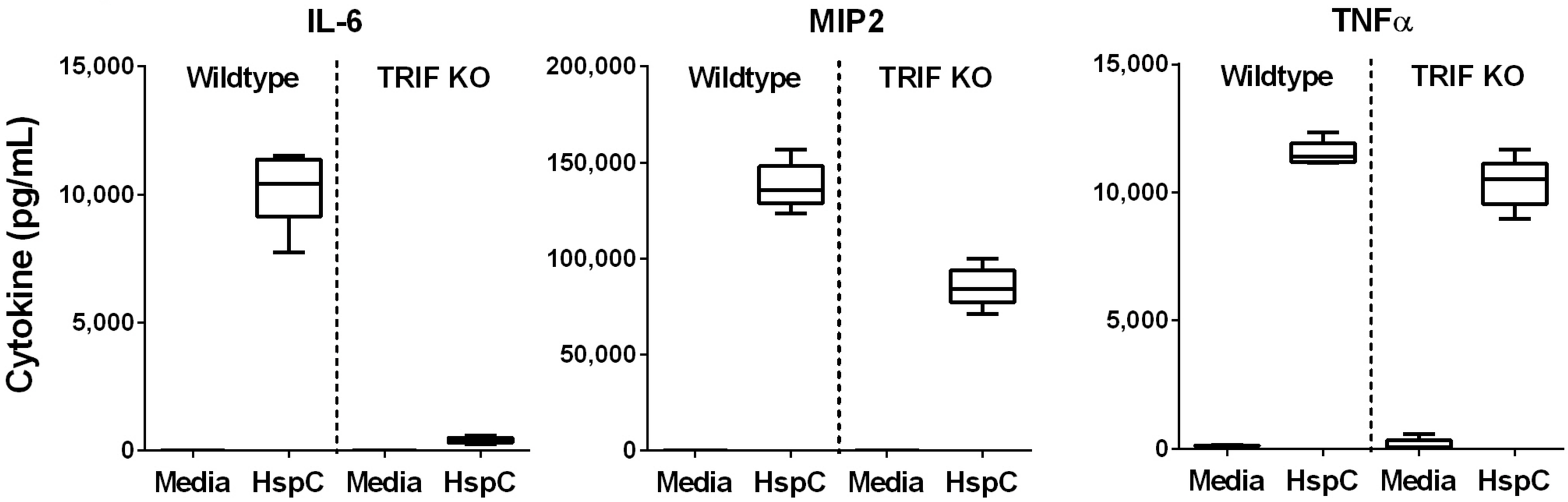
Lack of evidence for T-independent antigen response in neisserial HspC vaccinated mice

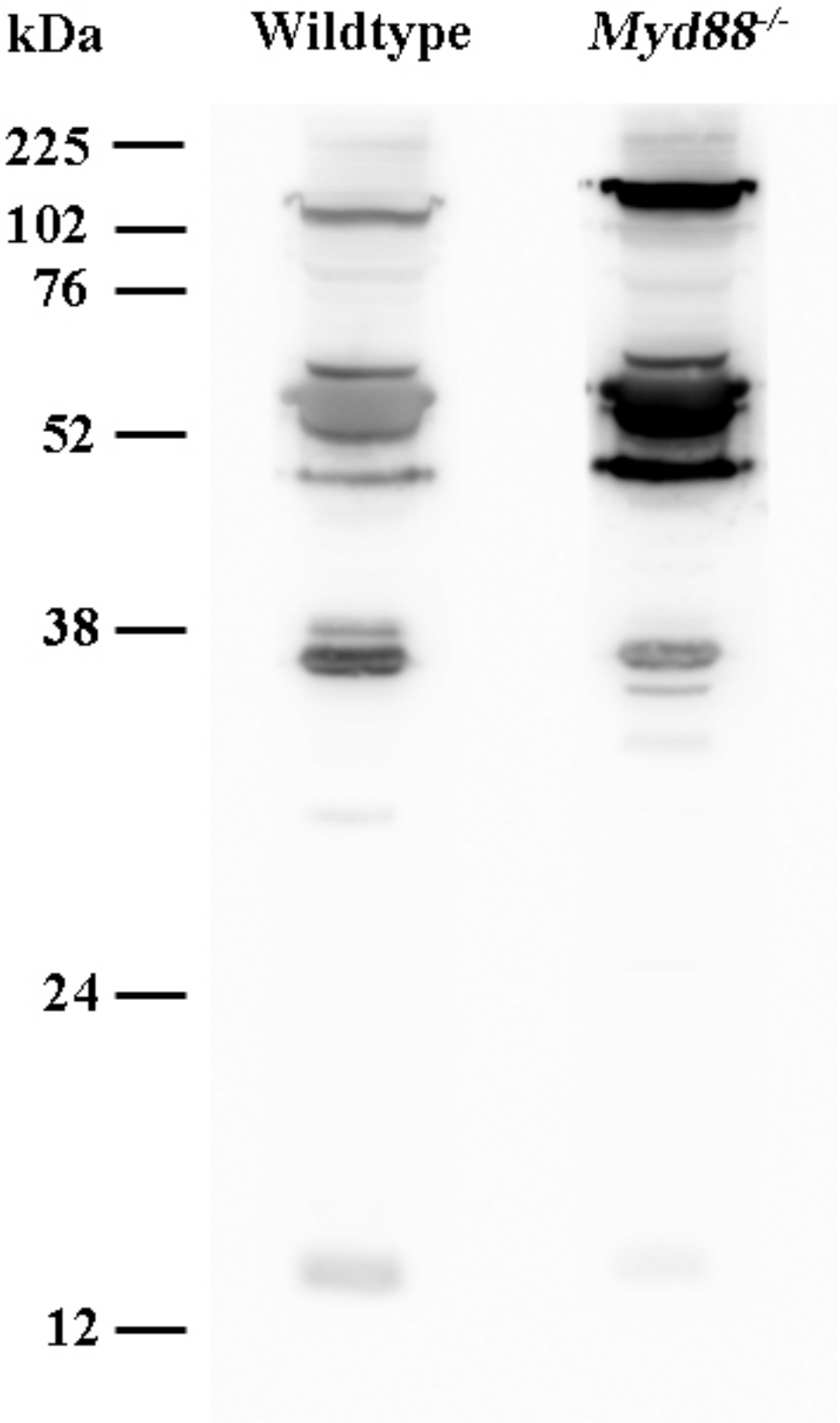
To evaluate whether the humoral response observed in HspC-vaccinated *Myd88*$^{-/-}$ mice in our published study might be due to a T-independent antibody response, sera from wildtype and *Myd88*$^{-/-}$ mice vaccinated with *N. meningitidis* HspC (collected from the experiment presented in Figure 4, ref [6]) were analyzed for immunoreactivity with HspC antigen by western blot (using technique modified from[14]).

Experiment 1



Experiment 2




kDa
Wildtype
Myd88-/-
225
102
76
52
38
24
12