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The formation of P particle increased immunogenicity of norovirus P protein

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Summary

As a commentary on a recently published paper in *Immunology*, this article summaries the principle of norovirus P particle as a promising vaccine against noroviruses. It emphasizes the importance of P particle formation in the immune enhancement of the vaccine and methods for production/verification of high quality P particles which may be easily neglected by researchers.

Keywords: antigen presentation; calicivirus; norovirus; P domain; P particle; vaccine; vaccine platform

The article by Tamminen *et al.*[1] performed a study to compare the immunogenicity of norovirus GII.4 virus-like particles with that of P particles in a mouse model. The authors concluded that the P particles induced a weaker, unbalanced T helper type 1/type 2 immune response compared with that of the virus-like particles and that the P particles did not induced cross-reactive B-cell and T-cell responses against different norovirus genotypes. However, this study seemed weak for evidence on efficient P particle formation in the materials used in their study and as a result the conclusions appear questionable.

Norovirus P protein expressed in *Escherichia coli* forms different P domain complexes, including the P dimer,[2–9] the 12-mer small P particle[10] and the 24-mer P particle,[11–13] which differ in their immunogenicity.[12] An important factor affecting P particle formation is the end-linked cysteine-containing tag that stabilizes the P particles through inter-P dimer disulphide bonds.[13] On the other hand, a P protein without an end-linked cysteine forms mainly P dimer.[4–9] Furthermore, addition of a non-cysteine-containing peptide to the ends of P domain significantly decreased P particle formation.[2,11,13]

In their article, Tamminen *et al.*[1] produced the P protein of a GII.4 norovirus by fusion of a His-tag to the C-terminus of the P domain[14] without a cysteine-containing tag. The evidence for P particle formation was an electron micrograph image without further biochemical validation, which was far from sufficient. First, the particles in the image seemed to be not structurally homogeneous and apparently differed from those formed by the P proteins with an end-linked cysteine tag reported previously.[11,13] Second, because a cysteine-containing tag was not included, the efficiency of the P particle formation of their P protein would be extremely low according to previous studies.[2,4–9,13] Hence, we are deeply concerned that the low immunogenicity of the so-called P particles in this study could be the result of a low efficiency of P particle formation or of different types of P domain complexes.

Before claiming the production of the same P particles as those reported previously, the authors need to follow the established procedures[13,15] and provide necessary evidence to support their claim. It has been found that the P protein without or with a non-cysteine-containing tag purified from an affinity column, e.g. the P protein with the His-tag of this study,[1] contains mainly P dimers with only a small portion of P particles[4–9,12,13,15,16] and a gel filtration analysis would give the P dimer/P particle ratio based on the molecular weights of the two P complexes ($\sim$ 70 000 and $\sim$ 830 000 Dalton, respectively). Unfortunately, this simple but critical experiment was missing from the study.

In summary, the observed weak immune response could be the result of the low immunogenicity of the materials that might mainly contain the P dimer. It is highly recommended that future studies pay attention to

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the principle of P particle formation through the cysteine-containing tag[11,13] and follow the established procedures to produce highly stable P particles. In addition, the established gel-filtration analysis has been proven to be a reliable approach to monitor the efficiency of the P particle formation of resulting P protein. As shown previously, the formation of P particle increased the immunogenicity of norovirus P protein significantly.[12]

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