Analysis of the vaccine-induced immunity against gastrointestinal parasites suggest a crucial role for mucosal IgG1 and memory NK cells

González-Hernández Ana1; Van Coppenolle S.1; Peelers I.1; Claerebout E.1; Geldhof P.1

1Faculty of veterinary medicine, Department of virology, parasitology & immunology. Salisburylaan 133, 9820 Merelbeke, Belgium.
ana.gonzalez@ugen.be

Introduction and aims

In recent years, our research group has developed protective experimental vaccines against the two most important gastrointestinal nematodes in cattle: the abomasal nematode Ostertagia ostertagi and the intestinal nematode Cooperia oncophora, based on activation-associated proteins (nASP) purified from the excretory-secretory material of adult worms. However, mimicking this response with recombinantly Pichia pastoris produced antigens (pASP), an absolute requirement for further commercialization, has been unsuccessful. The aims of our study were therefore to analyze and compare the immune mechanisms induced by the protective (nASP) and non-protective (pASP) vaccines against the gastrointestinal parasites Ostertagia ostertagi and Cooperia oncophora in both cattle and mice.

Results and conclusions

1) Antibody responses in cattle: Importance of mucosal IgG1

Mucosal IgG1

Ostertagia ostertagi

Cooperia oncophora

Systemic

Mucosal

2) Cellular vaccine-induced responses in cattle: A role for mucosal memory NK cells

Experimental setup

All animals were vaccinated intramuscularly 3 times in a 3-week interval with either 5 µg/ml antigen + 20 µg QuilA (mice), or 30 µg/ml antigen + 750 µg QuilA (cattle).

Mice were euthanized one week after last injection while cattle underwent infection for 5 weeks before euthanasia.

Systemic cellular response

Blood was taken weekly during the vaccination period and mononuclear cells (MNCs) from each group were analyzed for their proliferative capacities. To analyze proliferation systemically, MNCs were re-stimulated with nASP for 4 days, after which they were pulsed with 3H-Thymidine and measured in a scintillator 18h later.

As can be seen from the upper graphs, proliferation increased significantly after the second booster vaccination with nASP, and increased exponentially during the rest of the vaccination period in the case of C. oncophora. For O. ostertagi, although proliferation decreased, protection after nASP vaccination was still observed after challenge infection, which was not the case in animals vaccinated with pASP. This results indicate that there is a clear induction of immune memory following vaccination with native antigens.

Mucosal cellular response

After observing that protection was linked not only to an antibody response, but also a cellular response, we aimed to identify the cell type involved in this process. To do so, MNCs isolated at time of necropsy from the abomasal or small intestinal mucosa were stained with the membrane dye PKH26, stimulated with nASP and left in culture for 5 days. Afterwards cells were stained with monoclonal antibodies against specific markers for the different cell types (CD3, TCRg, CD3 and CD335).

Surprisingly, NK cells were the only cell type that responded following re-exposure with the O. ostertagi vaccine. The C. oncophora vaccine on the other hand induced both memory NK cells, B cells and T cells.

3) Vaccine-induced immune response is conserved among species: The use of mice as a model

Finally, aiming to analyze if the vaccine-immune response was conserved among species, we injected mice with O. ostertagi and C. oncophora native and recombinant antigens and analyzed both their antibody and cellular responses. Experiments were performed as for cattle.

Antibody response: As observed in cattle, vaccination with native and recombinant antigens induces specific IgG1 antibodies against nASP in blood. Since mice cannot be naturally infected with these parasites, mucosal information was not obtained.

Cellular response: Significant proliferation of MNCs of pASP and nASP vaccinated animals was observed after re-stimulation with the antigens. As observed in cattle, the response induced by pASP was lower and the cells involved were the same as those in cattle, being NK cells for O. ostertagi, and NK cells, B cells and T cells for C. oncophora. An additional cell type, CD3 CD335 CD21, also proliferated markedly after vaccination with pASP and nASP of both parasites.