Analysis by Radio-Immunoprecipitation Assays of the Antibody Response to Bovine Respiratory Syncytial Virus-Proteins in Experimentally and Naturally Infected Cattle

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Introduction

Infections with bovine respiratory syncytial virus (bovine RSV), are an important cause of respiratory tract disease in cattle. Signs may vary from subclinical to severe respiratory disease and death. Usually, calves of 1–3 months of age are most severely affected in outbreaks of bovine RSV-associated respiratory disease.

The genome of human RSV codes for ten unique viral proteins.[3] Four structural proteins are associated with the envelope of the virus, of which the fusion (F)- and the large glyco (G)- protein are the glycosylated surface proteins. The nucleo (N)- and phospho (P)- proteins are, together with the viral RNA polymerase associated with the viral genome, constituting the viral nucleocapsid.

In general, the polypeptide composition of human RSV and bovine RSV is similar; only minor differences in molecular weight of corresponding proteins are found.[1] The human and bovine strains of RSV was demonstrated, that the two human RSV subtypes and bovine RSV strains can be distinguished mainly on basis of epitope differences on the G-protein.[6]

The antibody response to RSV infections in humans has been analyzed by a number of workers.[2,7,8] Ward and coworkers demonstrated, that sera from human RSV infected infants were mainly directed to the N- and F- protein, while sera from adults, in addition, recognized the G-protein.[8] Vainionpaa and coworkers reported, that the antibody response in primarily infected humans was predominantly directed to the F- protein and the virus matrix (M)- protein.[7] Gimenez and coworkers detected mainly antibodies to the F- and N- protein in the sera from 33 human RSV infected humans.[2]

In this presentation, we report on the analysis by radio-immunoprecipitation assays of the antibody response in bovine RSV infected cattle. Sera from experimentally infected calves and from naturally infected calves, with or without maternal antibodies in their serum at the onset of disease, and from calves that died during outbreaks of bovine RSV associated respiratory disease, were analyzed.

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Materials and Methods

Growth and radiolabelling of cells and virus

The Lelystad strain of bovine RSV was grown on bovine fetal trachea (BFT) cells. Therefore, subconfluent monolayers of BFT cells were inoculated with a stock preparation of the virus. When the cells showed the first signs of cytophatic effects, usually three days after infection, medium was replaced with label medium containing either [^{3}H]glucosamine or [^{3}H]amino acids. After 48 hours of labelling, cells were collected and further processed for radio-immunoprecipitation.

Lysis of radiolabelled cells

Two slightly different lysis procedures were used. [^{3}H]glucosamine labelled cells were lysed in a buffer containing 1% Nonidet, whereas [^{3}H]amino acid labelled cells were lysed in a buffer containing 1% Triton X-100, 1% sodium deoxycholate and 0.1% sodium dodecyl sulphate (SDS).

Radio-immunoprecipitation assay

For radio-immunoprecipitation (RIP), lysates were incubated with a test serum and, subsequently, with a rabbit anti bovine Ig serum. Precipitates were collected, washed and then analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli.[5]

Tested sera

The sera were collected from cattle in outbreaks of bovine RSV associated respiratory tract disease. Diagnosis

was made with a previously reported method.[4] Sera were collected from calves, that did contain serum maternal antibodies to bovine RSV at the moment of disease outbreak, and from calves that did not contain maternal antibodies. Furthermore, sera were collected from calves, that died during bovine RSV associated disease outbreaks. Also sera from experimentally infected calves were examined.

Results and Discussion

With the RIP-procedure, using the two differently prepared lysates, we were able to identify the major bovine RSV proteins, ie, the G-, F-, N-, M- and phospho-protein, on basis of the reported molecular weights of the corresponding human RSV proteins.[3]

The F- and N- proteins appeared to be the most immunogenic bovine RSV proteins, antibodies directed to these proteins were detected in sera of experimentally and naturally infected calves. The G-protein is less immunogenic, antibodies to this protein were only detected in calves with maternal antibodies and in experimentally infected calves after challenge. No clear qualitative differences were found between the RIP-patterns obtained with the different categories of sera. That is to say, similar RIP-polypeptide patterns were obtained with sera of calves that died during a bovine RSV associated outbreak of respiratory tract disease, and with sera of calves that recovered from the disease. In general, all these sera contained neutralizing activity in a virus neutralization assay. Apparently, a fatal outcome of a bovine RSV induced respiratory tract disease, is not directly due to an incomplete, insufficient antibody response to bovine RSV proteins.

Summary

The specificity of the antibody response in several categories of cattle infected with bovine respiratory syncytial virus (bovine RSV) was analyzed. Therefore, two radio-immunoprecipitation assays were developed, one for the analysis of the response to the glycosylated envelope proteins, and the other for analysis of the response to the other viral proteins. The antibody responses in experimentally and naturally infected cattle, with or without maternal antibodies to bovine RSV were analyzed. The antibody response in naturally infected calves that recovered from disease, was compared with the response in calves, that died from disease.

References

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