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CYTOTOXIC T-CELL RESPONSE IN LAMBS VACCINATED WITH BOVINE RESPIRATORY SYNCYTIAL VIRUS

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ABSTRACT

Lambs inoculated intratracheally (IT) and subcutaneously (SC) with bovine respiratory syncytial virus (BRSV) vaccine were evaluated for development of cell-mediated immune (CMI). Cytotoxic T-cell response showed better immune response in lambs, vaccinated IT. Cytotoxic activity of lambs immunized IT and SC with BRSV and neutralizing antibody values were investigated dynamically.

Key words: lambs, bovine respiratory syncytial virus (BRSV), cytotoxic activity, T-cell response.

Introduction

Bovine respiratory syncytial virus (BRSV) is an important agent in the pathogenesis of respiratory tract disease in calves (Larsen, 2000).

Cell-mediated immune response (CMI) in humans and animals plays a major role in the recovery from infection (Nash et al., 2015). Development of an illness after intranasal inoculation of live virus in the presence of pre-existing anti-BRSV-serum antibodies might be cited as an evidence (Wraith et al., 1979). Moreover, there is a poor relation between the level of neutralizing antibodies and the resistance-to that respiratory virus (Stott et al., 1984; Field and Smith, 1984; Ellis et al., 1990). Intranasal immunization with recombinant bovine respiratory syncytial virus also induced neutralizing antibodies (Schmidt et al., 2002).

In order to prove the role of cell-mediated immune response additional experiments in lambs have been carried out. The results of the BRSV – specific cytotoxic T-lymphocytes in peripheral blood and lungs are reported in this paper, thus, a more detailed characterization of the Bulgarian vaccine strain is achieved.

Materials and methods

Bovine respiratory syncytial virus. Vaccine strain "An-87" was attenuated in BHK21 tissue culture with titer $10^{5,5}$ $TCID_{50}/cm^3$.

Biological experiment. Experimental work was carried out in 8–12-week-old lambs, free of neutralizing antibodies to BRSV, The first group of ten lambs was inoculated intratracheally (IT) with 10 ^{5,5} $TCID_{50}$ of "An-87"; the second group received $10^{5,5}$ $TCID_{50}$ of "An-87" subcutaneously (SC); the third group was a control one and the lambs were injected SC with non-infected tissue culture fluid (cultures of BHK_{21} cells were used for the growth of BRSV).

On 5, 10, 15, 20, 35 days after inoculation two lambs of each group were killed. Blood samples were collected with and without heparin, and lymphocytes were isolated from the lungs.

Lymphocytes. Peripheral blood samples were collected in heparin-coated tubes from lambs. Mononuclear cells were separated from diluted blood on Ficoll-Paque column (Pharmacia, Sweden). Cell viability was determined by the trypan blue exclusion technique. At the end cells was suspended in RPMI-1640 medium containing 10% fetal calf serum (FCS), 10 $\mu g/cm^3$ streptomycin and 50 U/cm^3 penicillin.

Lung lymphocytes were obtained by the method of Dimov et al. (1990).

Target cells. Monolayers of BHK_{21} cells were infected with BRSV. Uninfected control monolayers were also prepared. Twenty four hours after virus inoculation, both infected and control monolayers were harvested, washed and suspended in RPMI-1640 medium with 20% FCS in appropriate concentration.

Cell infected with a strain of Newcastle disease virus (NDV) were used as a control.

Cytotoxicity assay. Cytotoxicity assay was performed by a modified method of Kumagai and Ogra (1985). Briefly, 0.5 cm^3 of target cells were mixed with 0.5 cm^3 of effector cells in 24-well plates until reaching an effector: target cell ratio 100:1. Cell mixtures were incubated for 14 hours at 37°C in 5% CO_2. After that each well was washed three times and viable cells were detached with 0.5 % trypsin-EDTA. Cells were counted. Cell viability was determined by trypan blue. Cytotoxic activity of mononuclear cells was calculated according to the following formula: 100 – (number of target cells experimental well / number of target cells in control wells) x 100.

Data from the experimental trait were analyzed and represented as mean ± standard deviation (SD). Differences were considered statistically reliable at $p<0.05$.

Results

Table 1 shows cell cytotoxic activity of effector cells obtained from lambs vaccinated intratracheally. A better cytotoxic activity of lung effector cells is demonstrated. Both effector cells obtained from IT infected lambs lysed BRSV infected target cells. Uninfected cells or cells infected with NDV were not lysed.

Table 1: Virus specificity of cytotoxic lymphocytes obtained from lung and peripheral blood of 10 IT BRSV-vaccinated lambs on 5-day ($p< 0.05$)

TALGET CELLS (BHK_{21} CELLS)	EFFECTOR CELLS			
	LUNG CELLS		PERIPHERAL BLOOD CELLS	
	Control lambs	BRSV-infected lambs	Control lambs	BRSV-infected lambs
BRSV – infected	29.3±3.6*	7.1±1.6	19.5±0.4	4.1±0.37
NDV – infected	6.2±0.31	4.2±0.14	3.9±1.22	3.2±0.18
Uninfected	8.5±0.73	6.5±0.64	5.5±0.68	4.0±0.37

** mean percent lysis*

Fig. 1 shows the comparative results of cytotoxic activity of lambs immunized IT and SC with BRSV together with values of neutralizing antibodies. Cytotoxic activity of lung effector cells after IT immunization reveals higher values as compared to the SC application. The cell cytotoxic response is demonstrated earlier (peak on 5 day) than the serological response (peak on 20 day).

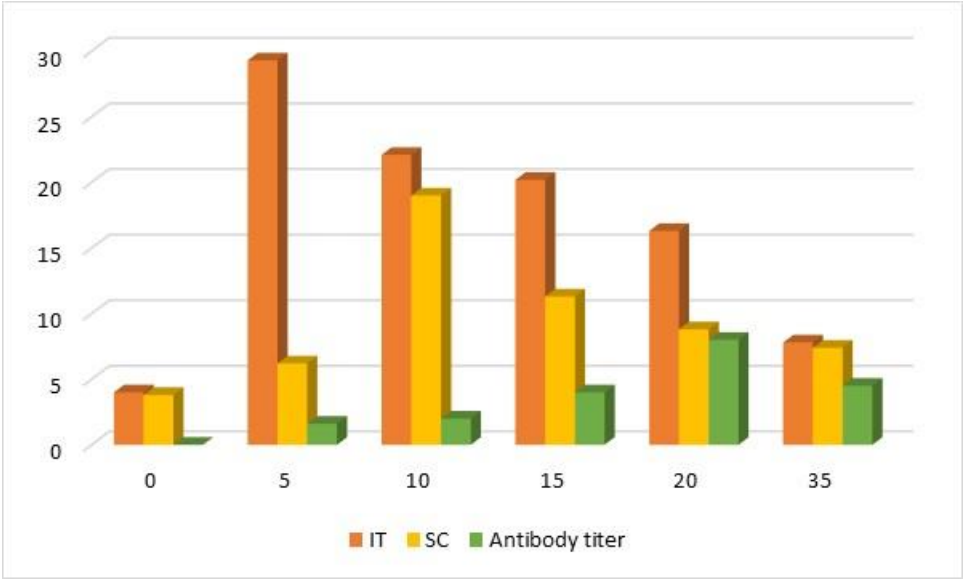

Figure 1: Comparative results of cytotoxic activity (%) and neutralising antibodies (log2) in lambs, vaccinated intratracheally and subcutaneously with BRSV

Discussion

Local, systemic and cell-mediated immunity as described in many reports develops after antigen stimulation in the respiratory tract. Immune response depends on thymus-dependent and thymus-independent lymphocytes (Schmidt, and Varga, 2018). These data are supported by the results of our experiments after vaccination with BRSV strain. Cytotoxic T-cell response in lambs with IT inoculated "An-87", is better expressed (29%) as compared to SC vaccination (19.5%). As Cole et al., (1973) have pointed out, this is possibly due to the direct effect of viruses on trachea and lung on one hand, and to sensitivity of cells, in which viruses have replicated, on the other hand. The highest values of cytotoxic response are estimated 5 days after IT, and 10 days after SC inoculation. Cytotoxic activity is virus-specific as neither uninfected nor infected with another virus cells (NDV) were lysed. Serum antibodies were determined later (peak on day 20).

In contrast, Kumagai and Ogra (1985) have reported that cellular cytotoxic reactivity is absent in cotton rats immunized SC with live human RSV. This possibility is due to the RSV replication in respiratory tract mucosa, follow intranasal inoculation. No virus replication is observed after SC inoculation of the virus. Like Schmidt suggested (2002) the BRSV attachment glycoprotein G is dispensable in vaccinating calves against BRSV.

Sharma and Woldehiwet (1991) have shorn a cytotoxic T-cell response in BRSV-infected 1ambs. Peak lymphocyte cytotoxic activity in peripheral blood and in spleen has occurred 10–14 days after infection. Regardless of the different dynamics our results together with the results of these authors prove the importance of cytotoxic response on one and the same model, i.e. lambs.

Sharma and Woldehiwet (1992) have reported that after experimental infection of lambs with BRSV, virus was isolated from nasal swabs and from the mononuclear fraction of peripheral blood. It is clear that exact mechanisms of that response are not well understood and results vary depending on animal species and virus strain.

Conclusion

The results show the importance of cell-mediated immune response after vaccination with live BRSV vaccine and the possibility that lambs could be used as a possible model for this type of infection.

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