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EXTRACTION OF ANTIGEN "A" FROM THE SKIN OF CHICKENS INFECTED WITH THE PATHOGENIC VIRUS OF MAREK'S DISEASE

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The paper presents possibilities for extracting antigen "A" from the skin; and feather follicle epithelium of chickens with Marek's disease virus MDV. The best method for releasing large quantities of MDV was sonicated destruction of the skin cells or extraction in sarcosyl buffer. All sera from infected chickens were positive in the agar gel precipitation (AGP) test with antigen "A" prepared in the laboratory and with reference antigen 0157/03(Weybridge). Further work is necessary in order to purify antigen "A" for routine laboratory work and investigate cellular immunity.

Key words: Antigen "A", virus, immuniti, Marek's disease

INTRODUCTION

Chubb and Churchill (1968) demonstrated the presence of 6 antigens in an extract of kidney cell culture infected with Marek's disease virus (MDV) using an agar gel precipitation test (AGPT). However, only one of those antigens was soluble and could be demonstrated in the supernatant of cell cultures. That was labelled antigen "A" and it was demonstrated that the antigen disappeared after attenuation of the pathogenic MDV (Churchil) et al., 1969). Soluble antigen "A" could be extracted from epithelial cells of feather follicles of chickens infected with MDV. Many attempts to purify and separate this glycoprotein have been described (Ross et al., 1975) and then it was found that its molecular weight is about 70.000 - 90.000 (Ross and Biggs, 1973). The antigen sediments at 3,758 to 4,28, it is stable at pH 2 and its isoelectric point varies from 4,5 to 5,35. Apart from this glycoprotein there were others found: gpD, gpI, gpE (Brunovski et al., 1992).

Antigen "A" is used in the AGPT for demonstration of the presence of antibodies to MDV. Recently, it was reported that imported antigens have been applied in our country in the AGPT for MDV (Rusov et al., 1992). However, due to difficulties in importation it is not possible nowadays to count on their routine application.

This paper reports on the extraction of antigen "A" from the skin and follicles of experimentally infected chickens with the aid of four preparative methods. At the same time, this is the first report about the preparation of this kind of antigen in our country.

MATERIAL AND METHODS

Virus. Marek's disease virus was isolated from a culture of chicken fibroblasts out of the blood of a diseased hen. Extracts of tumor and whole blood from the same hen were also used for infection of chickens. In a previous experiment the virus caused 75% mortality of chickens with the characteristic clinical and pathoanatomic picture of Marek's disease (MD) Gagić et al., 1993).

Preparation of antigen. A total of 30 one day old broiler chickens were used for infection with pathogenic virus. Three experimental groups were formed: group A with 10 chickens which were infected on the first day of life i/per with 0,2 ml of whole blood from the diseased hen. Group B with 10 chickens which were inoculated i/per by injecting 0,2 ml of suspension of tumorous cells from the same hen. Group C consisted of 10 chickens inoculated i/per with a suspension of a cell culture of chicken embryonal fibroblasts (CEF) with expressed cytopathogenic changes (CPC) typical for MDV.

Two chickens from each group were sacrificed in order to provide samples of skin and feather follicles and so that it could be established which of four preparative methods gave the greatest amount of antigen. A sample of 10 g of skin was taken from each chicken from the neck, chest, back and from the legs. Skin was cut using sterile scissors and divided into 4 equal parts. Each part was further processed according to one of the four preparative procedures described:

a) Extraction in PBS:

One part of the skin was treated 1:1 with PBS (pH 7,2-7,4). The tissue was homogenized using a "Vitris 45" apparatus 6 times for 30 seconds with 1 minute pauses. During the treatment the container with skin was surrounded by an ice bottle. After homogenization the extract of skin with follicles was filtered through ten-ply sterile gauze and divided into three portions.

The first portion of the extract obtained was marked (I) and was used as the crude anatigen.

b) *Extraction of antigen by freezing and thawing (II):*

The second portion of extract was frozen and thawed ten times at -10 C.

c) Extraction by ultrasound (III, IV):

The third portion of extract marked (III) was treated by ultrasound at 20 kc for 2 minutes with pauses after every 15 seconds. The container with antigen was surrounded by an ice bottle during the freatment.

A fourth portion (IV) of the antigen prepared in this way was centrifuged at 12500 rpm for 60 minutes at +4 C.

d) Extraction in Sarcosyl buffer (VI):

Skin with follicles was extracted in this buffer in the ratio 1:10 and homogenized in the same apparatus as described. The sample was then filtered through ten-ply sterile gauze. To each ml of this extract was added 0,03 ml of 1 N HCl (pH 4,0) and the mixtureput into a water bath for 1 hour. After centrifugation at 2500 rpm for 15 minutes the supernatant was removed and used as an antigen marked (V). The sediment was separated on filter paper and mixed with 0.1 ml of glycine buffer 0,1 M. This was thoroughly homogenized and used as a second portion of antigen marked (VI).

Determination of antigenic ability for precipitation

Antigenic ability was determined using an agar gel immunodiffusion test. Agar gel for this purpose was prepared as follows: 7,5 g of glycine and 5 g of NaCl p.a. were dissolved in 90 ml of redistilled water. Then 0.1 g of sodium azide and 2,0 g of Noble agar were added and the volume made up to obtain 100 ml. with redistilled water. The agar was then melted and poured out into Petri dishes for immunodiffusion (60 mm in diameter) or on to glass slides and perforate leaving wells 3 mm away from one another.

Specific antisera used in the test were obtained from chickens which had survived 90 days after infection with pathogenic MD virus. Antiserum 0157/3 obtained from Weybridge was used as the control serum.

The AGPT was performed as follows: dilutions of antigen starting with concentrated extract up to a dilution of 1/32, were put clockwise into earlier prepared wells in the agar gel in the form of a hexagon with one well in the centre. The central well contained a specific antiserum produced in the laboratory of the Veterinary Institute in Novi Sad (AsMD). Antiserum from Weybridge was put in another Petri dish with agar gel. Both Petri dishes were put in a humid chamber for the next 72 with daily observation for precipitation lines. Definitive results were noted on the third day.

RESULTS

All antigenic preparations obtained in these examinations gave a precipitation line in agar gel both with the reference antiserum and with the newly produced antiserum, respectively. The most marked precipitation line was obtained with antigen extracted by ultrasound extracts III and IV, as well as with antigen obtained in Sarcosyl buffer.

Table 1. Results of an examination for antigen"A" obtained from chicken skin by different preparative methods, three weeks after infection with MD virus

Infected chickens		Mode of extraction of antigen "A" Three weeks after infection					
Group	Number	I	II	III	IV	V	VI
A	1	—	conc	1 : 8	1 : 4	1 : 2	1 : 2
	2	—	conc	1 : 4	1 : 4	1 : 2	1 : 2
B	3	—	conc	1 : 8	1 : 4	1 : 2	1 : 4
	4	1 : 2	1 : 2	1 : 16	1 : 8	1 : 4	1 : 4
C	5	conc	1 : 2	1 : 4	1 : 4	1 : 2	1 : 2
	6	conc	1 : 2	1 : 2	1 : 4	1 : 2	1 : 2

Table 2. Results of an examination for antigen "A" obtained from chicken skin by different preparative methods five weeks after infection with MD virus

Infected chickens		Mode of extraction of antigen "A"					
		Five weeks after infection					
Group	Number	I	II	II	IV	V	VI
A	7	conc	conc	1 : 2	1 : 2	conc	conc
	8	NT	NT	1 : 4	1 : 2	NT	NT
B	9	conc	1 : 2	1 : 8	1 : 2	1 : 2	1 : 4
	10	NT	NT	1 : 4	1 : 2	NT	NT
C	11	conc	1 : 2	1 : 2	1 : 2	1 : 2	1 : 2

Tables 1 and 2 clearly show that there were differences in antigenic ability depending on the mode of extraction and the way of infecting the chickens. The highest titre (1:16) was obtained from a skin and feather follicle extract from chickens infected with a suspension of tumorous cells (Group B).

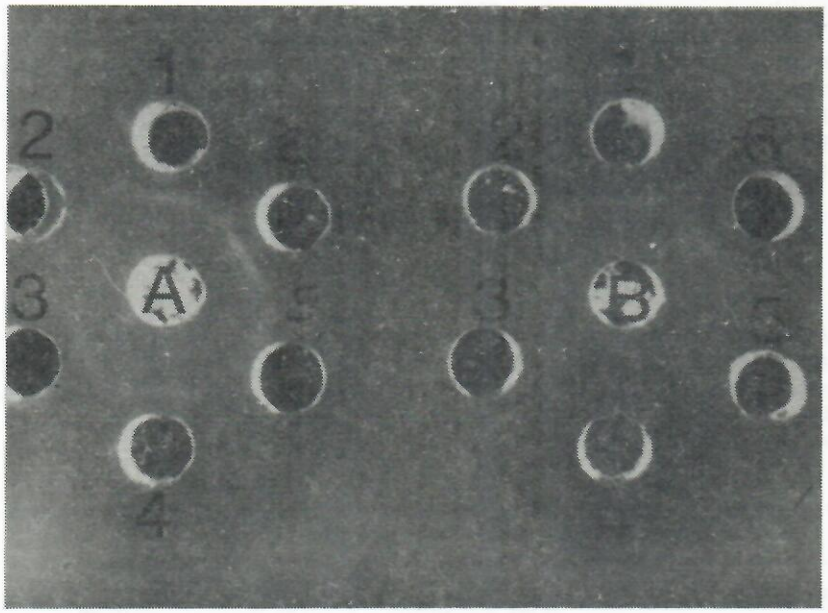

Figure 1.

a) A antigen MNS/91 (III)
1 antiserum 0156/3 Weybridge
3-6 antisera of chickens from the experiments

b) B antigen 0157/03 Weybridge
1 antiserum 0156/03 Weybridge
3-6 antisera of chickens from the experiments

Infection of chickens with blood from the diseased hen also gave us an extract which had good antigenic ability, while the weakest antigen was obtained from the skin and feather follicles of chickens infected with MD virus in cell culture. All sera gathered from surviving infected chickens were positive in agar gel both for the produced and reference antigen, respectively.

DISCUSSION

From the results obtained it follows that the procedures described enable production of the antigen "A" of MD virus. That antigen detected specific antibodies individually in AGPT both in examined and in reference sera.

Antigen "A" was obtained in other laboratories too, either from feather follicles (Haider et al., 1972), or from skin homogenate (Purchase et al., 1971) of chickens infected with MD virus. Applying the experience gained by other authors we tried to obtain antigen "A" using six preparative methods which seemed suitable for this purpose. On that occasion it was found that the best antigen was obtained when a maximal quantity of virus was released from the cell. The same was also achieved either by ultrasound disintegration of skin homogenate or by destruction of skin cells with Sarcosyl buffer.

The results obtained encourage the further aim of our work to reach a final purified product which would in routine work give even better results. Purification of antigen "A", as well as other components of MD virus may create conditions for their examination within the cellular immunological response process.

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EKSTRAKCIJA "A" ANTIGENA IZ KOŽE PILIĆA INFICIRANIH PATOGENIM VIRUSOM MAREKOVE BOLESTI.

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SADRŽAJ

U radu je opisana mogućnost ekstrakcije "A" antigena iz kože i ćelija epitela folikula pera od pilića inficiranih MB virusom. Najbolji metod za oslobađanje većih količina virusa MB bio je destrukcija ćelija kože ultrazvukom ili ekstrakcija u Sarkoksil puferu. Svi serumi inficiranih pilića bili su pozitivni u AGP testu sa "A" antigenom pripremljenim u laboratoriji kao i sa referentnim antigenom 0157/03 (Weybridge). Potrebna su dodatna ispitivanja u nameri da se prečisti "A" antigen za rutinski laboratorijski rad i ispitivanje celularnog imuniteta.