ACTA VET. BRNO, 56, 1987: 449-453

ANTIBODY RESPONSE IN DOMESTIC FOWL AS AFFECTED BY DIFFERENT IMMUNIZATION METHODS

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Received July 4, 1987

Abstract

Petrovský E., J. Kalová, V. Benda: Antibody Response in Domestic Fowl as Affected by Different Immunization Methods. Acta vet. Brno, 56, 1987: 449-453.

The differences are described in this paper as observed in the formation of antibodies in adult hens following application of different routes of immunization and using two antigens distinguished by different dependences on the thymus, actually the SRBC and B. abortus. Formation of the agglutination antibodies to SRBC was inferior to the latter, the i.m. immunization method of antigen application with adjuvant, in conjuction with simplicity of the procedure, in general leading to optimum titres of specific antibodies.

Antibody response, immunization method, domestic fowl.

The studies concerned with the immunological profile of farm animals are motivated primarily by efforts aimed at amelioration within the frame of selection programmes, not only the commercial traits but also the fitness and natural resistance to negative influences of the environment (Gavora and Spencer 1982). Information of extraordinary importance is furnished by the data on the formation of specific antibodies following immunization with natural, multideterminant antigens, such as erythrocytes or microorganisms. In this sphere, a number of conclusive differences have also been observed in chicks (Van der Zijpp 1983) where, besides the individual characters of the receptor, such as its age, sex, and individual degree of immunoreactivity, it is possible to markedly influence the dynamics of antibody formation by the method of immunization used.

In this paper are described the differences observed following the application of "thymus-dependent" (sheep red blood cells -

- SRBC) or "thymus-independent" (Brucella abortus) antigens in the experimental material used.

Materials and Methods

Experimental animals

The hens used for immunization were from a synthetic strain obtained by a diallelic crossing of 2 WL lines, 1 RIR line and 1 RIW line, all aged 14 months. Three groups of full-sibs were made with 8 individuals in each.

Immunization procedure

The experimental birds were immunized using two antigens, namely the SRBC and B. abortus, strain S99 Weybridge, devitalized with heat (BIOVETA, Ivanovice na Hané, Czechoslovakia). Both antigens, concentration 5 % in PBS, were mixed to make a volume of 1 ml and then administered at the same time: intravenously (i.v.), intraperitoneally (i.p.) or, following their mixing with the same volume of incomplete adjuvant (BIOVETA), also intramuscularly (i.m.), always at 10-day intervals. The level of antibodies was determined in the plasma sampled using heparin to that end, on 10th day (first sampling) and on 15th day (second sampling) following the first immunization by agglutination in microtitre plates (U bottom) and test tubes (SRBC and B. abortus, respectively). The titre was expressed as log of reciprocal value of the highest dilution at which the agglutination still occurred.

Statistical assessment

The differences found in the studied groups of birds concerning the formation of antibodies were assessed statistically using the analysis of variance (antigen, immunization method, and time of sampling as fixed effects) and the t-test. The correlations between these parameters were assessed, in addition, using the coefficients of determination; their significance was tested by the F value.

Results

Individual groups of birds showed a number of differences in both the dynamics and titres of antibodies with respect to the antigens used (Fig. 1). It was possible to demonstrate that the experimental birds responded better to B. abortus (the average titre of all three immunization procedures following 1st and 2nd sampling was 2.47 and 2.40, respectively) than to SRBC (1.46 and 1.63, respectively). In either antigen the antibody response was affected by the method of application, too. Thus in B. abortus this factor together with replication of the immunization took a share of 19.7% in the variability of the final level of antibodies, but the effect was not significant. In the case of SRBC,

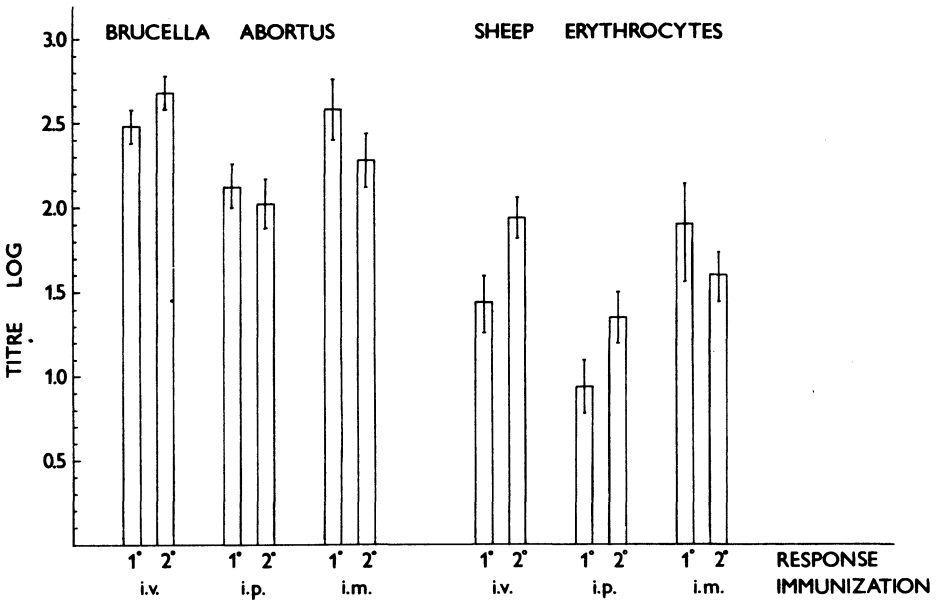

Fig. 1. Antibody titres to Brucella abortus and sheep erythrocytes (mean ± S.E.M.)

on the other hand, the share of its effect amounted to 38.6% and, similarly as that of the immunization method alone (26.5%), was at a level of high significance (Tab. 1). The i.p. mode of application appeared to be least effective for either antigen, without any more prominent differences in remaining procedures being also the order of sampling.

Table 1

Variance analysis of total antibody response

Source of variability	Brucella abortus		Sheep erythrocytes	
	Determination ratio η^2 %	Value F	Determination ratio η^2 %	Value F
Injection route	11.47	2.85	26.48	8.61^{x}
Response	0.95	0.47	2.58	1.68
Interaction	7.25	1.80	9.49	$3.09^{(x)}$
Total	19.67	1.95	38.55	5.01^{x}

s - significant at P = 0.05

The coefficient of determination (Tab. 2) furnished important information on the differences in the methods of immunization. In the relation between the antibody level to B. abortus and to SRBC, high values were observed only with the i.m. administration (a total of 0.53). Similarly, the values determined for relations

between the titres after 1st and 2nd samplings made following the i.m. immunization were at a level of significance in either antigen (0.72 for B. abortus, 0.81 for SRBC), and also in the latter following the i.v. administration (0.60). The antibody response to either antigen revealed lowest variability with the i.v. administration; in the case of SRBC, reduced variability in secondary responses was recorded for all instances involved.

Table 2

Correlation between antibody titres to BA and SRBC (r^2/F)

Injection	Response	BA/BA	SRBC/SRBC	BA/SRBC
I.v.	1^o	0.058/0.37	0.597/8.89^x	0.315/2.76
	2^o			0.008/0.04
I.p.	1^o	0.346/3.17	0.054/0.34	0.245/1.94
	2^o			0.015/0.09
I.m.	1^o	0.718/12.75^x	0.805/20.68^x	0.572/6.68^x
	2^o			0.529/5.61$^{(x)}$

Discussion

Selection of convenient immunization scheme, and in particular of the method of antigen administration is an essencial precondition for the study of immune response. These, in the cases of antigens used in our experiment, are affected by different factors.

Van der Zijpp et al. (1986) observed the effect at the level of significance due to the mode of antigen administration in SRBC, but not in B. abortus. The results of our experimentation were in support of this finding, despite the fact that the experimental birds responded with antibody titres that were markedly lower, in all probability due to the different concentration of SRBC and the higher age of chicks. In the study of Van der Zijpp et al. (l.c.) even the difference in age embracing several days played a role with the chicks subject to immunization. Superior antibody responses in older animals (parents at 20 weeks of age) compared to the chickens (offspring at 8 weeks of age) were observed by Gyles et al. (1986) - all these research findings indicating that the development of immune responses is a very complex phenomenon. It is known that during the first stage of the immune process in an organism, destruction of the antigen by macrophages and its presentation to B lymphocytes determine the degree of antibody formation. In the case of T-dependent antigens - as are those of SRBC - the immune response becomes affected distinctly by the administration of adjuvant. Concerning the reduction in secondary responses described by other authors, too (Ubosi et al. 1985), considered should be also the possibility of interaction between the antigens featuring different T-depen-

dences. The statistical assessment mentioned above clearly illustrates both the influence of immunization factors and the interdependence of the two antigens used.

The results obtained here suggest possibility and feasibility of the procedures of simultaneous immunization with the use of two antigens and create a background upon which the studies concerned with the immunological profile of chicks can be investigated further.

Protilátková odpověď kura při různých způsobech imunizace

Jsou popisovány rozdíly v tvorbě protilátek po různých způsobech simultánní imunizace dospělých slepic dvěma antigeny s odlišnou závislostí na thymu - ovčími erytrocyty a B. abortus. Lépe se tvořily aglutinační protilátky proti B. abortus, přičemž i.m. způsob imunizace s adjuvans - při technické jednoduchosti - obecně vedl k optimálním titrům specifických protilátek.

Отзывчивость антител на разные способы иммунизации

Описаны различия в образовании антител после разных способов симультанной иммунизации спелых кур двумя антителами, обладающими различной зависимостью от тима - посредством овчинных эритроцитов и *Brucella abortus*. Лучше образовались агглютинационные антитела против *B. abortus*, при этом внутримышечный способ применения с адъювантом - технически простой метод - в общем привел к оптимальным титрам специфичных антител.

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