Cross reactivity of deer immunoglobulin G (IgG) with antibovine IgG conjugate

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Abstract

Many approaches to elucidate the evolutionary relationship between ruminants and deer have been undertaken. We report a study conducted to find out the cross-reactivity of immunoglobulin G (IgG) of sambar deer (*Rusa unicolor*) and chital (*Axis axis*) to antibovine (AB) IgG horse radish peroxidase (HRP) conjugate by enzyme linked immunosorbent assay (ELISA). Various dilutions of bovine, sambar deer, chital and guinea pig sera were reacted against various dilutions of AB HRP conjugate and the optical density (OD) values noted. Results of the study indicated that there is cross reactivity between bovine and deer (sambar deer and chital) immunoglobulins which was evidenced by color development in ELISA. It was also noted that the AB HRP conjugate detected sambar deer IgG to a greater extent than chital IgG. However, on statistical analysis, it was seen that there was significant difference between the OD values. Hence it is concluded that there exists antigenic similarity between deer and bovine IgG and that even the sambar deer IgG differ antigenically from that of chital.

Keywords

Cross-reactivity, immunoglobulin G, sambar deer, chital, bovine, ELISA

Abbreviations

AB – Antibovine; BSA - Bovine serum albumin; ELISA - Enzyme linked immunosorbent assay; H_2O_2 - Hydrogen peroxide; H_2SO_4 - Sulphuric acid; HRP - Horse radish peroxidase; IgG - Immunoglobulin G; OD - Optical density; OPD - Ortho phenylenediamine dihydrochloride; PBS - Phosphate buffered saline; PBST - Phosphate buffered saline with 0.05% Tween 20

Introduction

Suborder *Ruminantia* under Order *Artiodactyla* has families *Tragulidae*, *Giraffidae*, *Cervidae* (deer), *Moschidae*, *Antilocapridae*, and *Bovidae* (antelopes, cattle, gazelles, goats, sheep, and relatives), and a number of extinct groups. These families present common anatomical characters like fused naviculars and cuboids, missing upper incisors to name few (Myers 2001). Cross-reactivity of immunoglobulin molecules of different species have been used as a criterion for finding out the phylogenetic relatedness between species (Nollens *et al.* 2008). In this paper, we describe the results of a study conducted to find out the cross-reactivity of IgG of sambar deer (*Rusa unicolor*) and chital (*Axis axis*) with that of bovines (*Bos indicus*) employing ELISA.

Materials and methods

Sambar deer, chital, bovine and guinea pig sera used in the study were from a small scale serum bank maintained in the Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Pookot. The serum from all the four species were tested separately against AB IgG HRP conjugates using ELISA. Bovine serum was used as the homologous serum (which binds maximally with AB conjugate and guinea pig serum as the heterologous one (which being very much different phylogenetically from bovines binds minimally).

Briefly the test was carried as per the procedure described below. Two fold dilutions of bovine serum starting from (1:1000 to 1:1,28,000) were made in carbonate – bicarbonate coating buffer (pH 9.6). Hundred microlitres each of 1:1000 diluted serum was dispensed in first five wells of the first column of an ELISA plate. Similarly the next dilution (1:2000) was dispensed in first five wells of the second column and so on till the last dilution (column eight). The plate was incubated overnight at room temperature in a humid chamber. After the incubation, the contents of the wells were discarded and the wells washed five times with PBS pH 7.4 containing 0.05% Tween 20 and tapped dry in lint free towel. The unbound sites in each well were blocked with 200µl of 1% BSA in PBS for 2 hours at 37°C and the plate washed and dried as before. Two fold dilutions of the AB HRP conjugate (starting from 1:1000 to 16,000) were prepared in PBST BSA (1% BSA in PBST) and 100µl each of 1:1000 dilution was loaded in the first 8 wells of the first row of the plate; 1:2000 dilution added in a similar manner to the second row and so on till the fifth row (1:16,000). The plate was incubated at 37°C for 1 hour, after the incubation, washed and dried as before. Hundred microlitres of 0.04% OPD in citrate buffer pH 4.0 containing 0.05% of 30 volume H_2O_2 was dispensed in all the wells in which the above reagents were added and the plate incubated for 10 minutes in dark at 37°C for color development. Then 100µl each of 1.25M H_2SO_4 was dispensed into all the wells to stop color development. The OD of each of the wells was read using an ELISA reader at 490 nm. In a similar manner, the experiment was done separately for sambar deer, chital and guinea pig sera.

The OD values for the serum dilutions (1:1000 to 1:28,000) of the four species for each dilution of AB conjugate (1: 1000, 1:2000 and so on) were analysed using single factor ANOVA to find out statistical significance. For each conjugate dilution, the OD values of bovine were compared with other three species *i.e.* the species pairs bovine/ sambar deer, bovine/chital and bovine/guinea pig, using student's t test (Zar 2010).

Results

The highest OD values for any dilution of serum/conjugate combination tested were obtained for homologous serum and conjugate *i.e.* between bovine serum/AB conjugate. As expected, guinea pig serum showed very little color development even at very low dilutions and hence negligible

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Table 1. Mean OD values for sera dilutions (1:1000 to 1:1,28,000) for various dilutions of conjugate and P values for comparison by single factor ANOVA

AB conjugate dilutions	1: 1000	1:2000	1:4000	1:8000	1:16000
Bovine	1.3381 [a]	0.6835 [a]	0.3981 [a]	0.2505 [a]	0.1561[a]
Sambar	0.4460 [b]	0.2298 [b]	0.1469 [b]	0.1089 [b]	0.0866 [b]
Chital	0.1171[c]	0.0888 [b]	0.0836 [b]	0.0763 [b]	0.0730 [b]
Guinea Pig	0.0890[c]	0.0765 [b]	0.0761 [b]	0.0740 [b]	0.0709 [b]
P value	2.05×10^{-11}	2.3×10^{-9}	1.25×10^{-8}	4.59×10^{-9}	1.35×10^{-8}

Data subjected to logarithmic transformation for statistical analysis. Means with different superscripts in a column are significantly different (P<0.05)

cross reactivity to the AB conjugate indicating it's dissimilarity to that of bovine IgG. Serum of sambar deer and chital showed varying degrees of cross reactivity to the conjugate as evidenced by varying color development. It was also noticed that the AB conjugate could distinguish sambar deer and chital serum at different levels. Sambar deer serum showed higher OD values than chital serum for corresponding dilutions indicating a greater cross reactivity with AB conjugate but statistically significant only at conjugate dilution of 1: 1,000 (Table 1).

Statistical analysis showed that there was significant difference (P<0.05) among OD values for the serum dilutions of the four species for a particular dilution of AB conjugate (Table 1). Bovine serum showed significantly higher OD values compared to the other three species sera in all dilutions.

Discussion and Conclusions

Various streams of research probing into the phylogeny of *Ruminantia* and *Cervidae* have been undertaken. These include studies employing inhibition of radioimmunoprecipitation of immunoglobulin antigens (Curtain and Fudenberg 1973) and the sequence analysis of mitochondrial control region (Allard *et al.* 1992). In this study by employing ELISA, evidences are been presented for cross-reactivity between IgG of two deer species with AB conjugate indicating antigenic similarity between the IgG of these three species. Though similar studies employing deer serum and AB conjugates have not been reported, the results agree with the findings of Cap *et al.* 2002 that *Bovidae* and *Cervidae* (deer) are sister clads based on behaviour.

Though the AB conjugate detected variable antigenic difference between the two deer sera under test, the comparable cross-reactivity of the IgG of sambar deer and chital with AB conjugate indicate the antigenic similarity of the molecule with bovine IgG.

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