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Production and Partial Purification of Anti-Species Antibodies

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ABSTRACT

In this study serum immunoglobulins (Igs) of the four species (human, buffalo, goat and chicken) were isolated and partially purified through ammonium sulphate precipitation technique. The amount of total protein in ammonium sulphate precipitated sera of the four species was found as 30.93, 45.37, 27.96 and 24.82 mg/ml. While the total protein in rabbit hyperimmune sera was ranged from 13.14 – 16.30 mg/ml with the mean of 14.86 mg/ml. Highest GMT (107.6) was observed in case of chicken followed by human (90.5), buffalo (76.11) and goat (64.0) anti-species antibodies. Multiple shot regimens gave significantly higher titres than the twin shot regimens and maximum increase was recorded in case of rabbit anti-buffalo Igs (2.38 fold).

INTRODUCTION

Enzyme linked immunosorbent assay (ELISA) is one of the most advanced, highly sensitive and very useful diagnostic procedure. There are various types, modifications and applications of the assay. It is used in epidemiological studies of various diseases, endocrinology, identification of tumor markers and detection of infectious diseases (Voller & Savigny, 1980). Among various types, indirect ELISA has proved to be very popular because a single methodology can be used to replace many tests for diagnosis of different diseases in a species. It has also been a method of choice to detect the presence of serum antibodies against Human Immuno deficiency Virus (HIV), the causative agent of AIDS (Kuby, 1994; Tortora *et al.*, 1995).

The most significant components of ELISA is anti-boidies-enzyme conjugate. The antibodies against an antigen are used in Direct ELISA whereas in case of Indirect ELISA antibodies produced against Igs of a species are applied. A number of enzymes are used for conjugation with the antibodies like alkaline phosphatase and horse radish peroxidases. This paper pertains to production of anti-species antibodies of human, buffalo, goat and chicken in rabbits. The optimized conditions for the standardization of Ager Gel Precipitation Test (AGPT) have also been discussed.

MATERIALS AND METHODS

Experimental Laboratory Animals. A total of 36 adult, male rabbits strain chinchilla were selected for production of anti-species antibodies. The animals were divided into five groups (A to E). The groups A-D were further subdivided into two subgroups, each sub-group of A-D and group E comprising of four rabbits.

Groups A-D were used for the production of anti-species antibodies against human, buffalo, goat and chicken, respectively, while the group E was maintained as non-inoculated (absolute) control. One subgroup was used for twin shot protocol while the other subgroup was inoculated through multiple shot regimen in each group.

Collection of human, buffalo, goat and chicken serum. An amount of 100 ml blood of each species was taken in a sterilized beaker. Human blood was collected from postgraduate students of the department of Biochemistry, University of Agriculture, Faisalabad. Blood of buffalo and goat was collected from animals of each species slaughtered at Faisalabad abattoir. The chicken blood was taken from chicken slaughtered at a broiler chicken shop in a glass beaker and allowed to clot. The oozed out serum was harvested in clean screw capped glass bottles which were placed under refrigeration till partial purification of Igs.

Partial purification of human, buffalo, goat and chicken Igs. Ammonium sulphate precipitation technique was applied for partial purification of human, buffalo, goat and chicken Igs following the procedure described by Hudson and Hay (1980). Igs were desalted by dialysis.

Inoculation of rabbits with species Igs. The rabbits were inoculated with species Igs through S/C route using twin and multiple shot regimen (Table I). **Collection of hyperimmune serum and isolation of rabbit anti-species (Igs).** Blood from rabbits of all the groups was collected by slaughtering the animals on the 28th day post first inoculation. On the next day the oozed sera was collected. The sera were processed for isolation of Igs through ammonium sulphate precipitation technique.

Estimation of protein in ammonium sulphate precipitated hyper immune sera. The amount of proteins present in the ammonium sulphate precipitated hyper immune sera of rabbits were measured using Biuret protein estimation method (Sheikh, 1991).

Titration of anti-species antibodies. The titre of anti-species antibodies was determined by AGPT (Hudson & Hay, 1981). The test was standardized using different grades of agar (noble agar, bactoagar and agarose), concentrations or agar (0.8, 1.2 and 1.6%), types of diluents (normal saline, phosphate buffer saline and vernal buffer) and incubation temperatures (4, 20 and 37°C) for each species. The wells of 6mm diameter were made with metallic tube attached with syringe through rubber tube. The bottom of the well was sealed with small drops of molten agar gel to prevent the reagent leakage under the gel. The pattern or wells around a central well was used. The petri dishes were placed under humid conditions to avoid drying of gel. The known species Igs were added in the central well, whereas rabbit-anti-species Ig(s) were added in the peripheral wells.

The rabbit hyperimmune serum sample having maximum protein after ammonium sulphate precipitate were

applied for the standardization of test making serial two fold dilutions from 1:2 to 1:256. The rabbit hyperimmune sera having 17.22, 17.41, 17.41 and 17.78 mg/ml protein were applied for human, buffalo, goat and chicken, respectively. Each plate was covered with filter paper, soaked in distilled water and placed in refrigerator upto the development of precipitation line. The conditions showing clear sharp precipitin band at earliest were taken as the standard conditions for the test. The reciprocal of the highest dilution of the rabbit anti-species Igs giving positive results through the standardized AGPT was taken as AGPT titer of rabbit anti-species Igs.

RESULTS AND DISCUSSION

Isolation of species Igs. The amount of total protein in ammonium sulphate precipitate sera of the four species was measured and found as 30.93, 45.37, 27.96 and 24.82 mg/ml of human, buffalo, goat and chicken serum, respectively.The observed values were relatively higher than reported values of the IgG in the four species. This increase might be due to some proteins other than IgG which could not be removed with the method applied. However, the findings were in accordance with the results of Ratyal *et al.* (1992), who reported 48 and 52 mg/ml of Igs in buffalo serum with 35 and 40% ammonium sulphate precipitation.

Rabbit anti-species Igs. Total protein ranged from 13.14-16.30 mg/ml with the mean of 14.86 mg/ml. In rabbits of sub-group A (Twin shot human), the mean concentration of total protein was 13.93 mg/ml showing an increase of 7.2% from the control. In case of rabbits inoculated by human Igs through multiple shot regimen, the increase in total protein was 11.17% with a mean concentration of 16.52 mg/ml. The increase in total protein of ammonium sulphate precipitate hyperimmune serum might be due to the increase in the amount of specific antibodies against the inoculum (species Igs).

The precipitin test gives a great deal of basic information, it is lengthy to perform and requires relatively large quantities of reagents. Many gel precipitation techniques have been developed to overcome the problems (Hudson & Hay, 1980). Simple diffusion (Qudin's technique) and the double diffusion (Ouchterlony's technique) are most common types (Malik, 1988). The method applied in the present study was Ouchterlonys technique in which antigen and antibodies are allowed to migrate towards each other in a gel and line of precipitation is formed where the two reactants meet (Kimball, 1983; Malik, 1988).

Noble agar and agarose with concentration of 1.2% in veronal buffer of pH 8.6 assured the best results for all the species. When three temperatures (4°C, 20°C and 37°C) were compared for incubation of the best, earliest (16 hours) results were observed at 4°C incubation, however, 20°C and 37°C also gave results at 20 to 24 hours post-incubation. The results revealed that these standardized conditions of the test can be adopted successfully for the qualitative and quantitative determination of anti-species antibodies. The AGPT has been recommended for detection of anti-species antibodies. by Hudson and Hay (1980). The agar gel precipitation test can also be applied for the conformation of infectious diseases like IBD (Aziz, 1988).

Table I. Inoculation schedule

Day of inoculation	Dose of inoculum (ml)	
	Twin shot	Multiple shot
0	1.7	0.2
2	-	0.4
5	-	0.6
8	-	0.8
11	-	1.0
14	3.3	2.0
Total	5.0	5.0

AGPT Titre of Rabbit Anti-species Antibodies. Highest GMT (107.6) was observed in case of chicken followed by human (90.5), buffalo (76.11) and goat (64.0) anti-species antibodies. Multiple shot regimens gave significantly higher titres than the twin shot regimens and maximum increase was recorded in case of rabbit anti-buffalo Igs (2.38 fold). In case of rabbit anti-human and rabbit anti-chicken Igs, increase of 1.41 fold was recorded in multiple regimen than the twin shot regimen. Whereas in case of rabbit anti-goat Igs the titre was exactly doubled in case of multiple shot protocol. No titres were observed in case of sera obtained from rabbits of control group when tested against species Igs of human, buffalo, goat and chicken.

When Igs of a species are inoculated into another species, the recipient species produces antibodies (anti-species anti-bodies) against the Igs of donor species. Greater is the difference between donor and recipient species, higher would be the titre of anti-species antibodies produced. The species difference between rabbit and chicken is greater than the difference between other species (human, buffalo and goat) and rabbit. The highest AGPT titre observed in case of rabbit hyperimmune sera against chicken might be due to this greater species difference.

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