Analysis of animal serum proteins using antisera against human analogous proteins

A study of immunological cross reaction between human and animal serum proteins

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INTRODUCTION

Qualitative and quantitative analysis of serum proteins most often requires the use of monospecific antibodies against the proteins in immunochemical assays such as gel immunodiffusion, gel immunoelectrophoresis, enzyme linked immunosorbent assays, radioimmunoassays and immunohistochemical assays.

Although specific antisera against many serum proteins of a large number of animal species have been raised and rendered commercially available, the most complete list of antisera have been developed against human proteins.

Fortunately evolutionary conservatism has resulted in common epitopes of many serum proteins between various mammalian species, and the aim of the present study was to analyse to what extent commercially available antisera against a number of human serum proteins could be used for the analysis of analogous animal proteins. It is well known that antisera against human serum proteins are applicable for the analysis of analogous proteins in closely related species, e. g. primates (*Bohn & Ronneberger* 1973, *Bohn & Sedlacek* 1975, *Hau* 1986) but even phylogenetically distant species may have similar epitopes present on their corresponding serum proteins which makes antisera against human analogues useful for the analysis of these proteins.

The assay we chose for the present analysis was rocket immunoelectrophoresis (*Laurell* 1972), because this assay requires an immunological cross reaction sufficient enough to result in precipitation between the antibodies against the human protein and the animal analogue protein. A cross reaction recorded in this system indicates that any other immunochemical system may also be successfully applied for quantitative as well as qualitative analysis of the animal protein in question.

MATERIALS AND METHODS

Antigens:

Serum from pregnant females of the following species was analysed in rocket immunoelectrophoresis:

Mouse (*Mus musculus*)
Rat (*Rattus norvegicus*)
Hamster (*Mesocricetus auratus*)
Guinea pig (*Cavia cobaya*)
Mink (*Mustela vision*)
Polecat (*Mustela putorius*)
Raccoon dog (*Nyctereutes procyonoides*)
Cat (*Felis domestica*)
Dog (*Canis familiaris*)
Cow (*Bos taurus*)
Pig (*Sus scrofa*)
Horse (*Equus caballus*)
Goat (*Capra hircus*)
Sheep (*Ovis aries*)
Red Deer (*Cervus elaphus*)
Wildebeest (*Connochaetes gnu*)
Musk ox (*Ovibos moschatus*)
Elephant (*Elaphas maximus*)

Seal (*Phoca vitulina*)
Hen (*Gallus bankiva*)
Monkey, marmoset (*Callithris spp.*).

Samples of pools of serum from pregnant women and men were analysed in all electrophoreses as positive controls.
All samples were analysed undiluted.

Antibodies:
The antibodies used were all from Dakopatts, Glostrup, Denmark. Antibody preparations raised in rabbits against the following human proteins were used:

Alpha-fetoprotein (AFP), code no. A008, lot no. 097
Human placental lactogen (hPL, hCS), code no. A137, lot no. 054A
Human chorionic gonadotropin (hCG), code no. A231, lot no. 085
Pregnancy specific beta-1 glycoprotein (SP1, code no. A131, lot no. 101
Corticosteroid binding globulin (CBG, Transcortin), code no. A298, lot no. 014
Pregnancy Zone Protein (PZP, alpha-2-PAG), code no. A132, lot no. 100
Pregnancy associated plasma protein-A (PAPP-A), code no. A230, lot no. 011
Orosomucoid (alpha-1-acid serum protein), code no. A011, lot no. 014
Alpha-2-Macroglobulin (alpha-2-M), code no. A033, lot no. 011
Prealbumin, code no. A002, lot no. 026
Transferrin, code no. A120, lot no. 063
Immunoglobulin G, code no. A090 (new code no. 423), lot. no. 062
Immunoglobulin M, code no. A091 (new code no. 426), lot. no. 078
Haptoglobulin, code no. A030, lot no. 016
Ceruloplasmin, code no. A031, lot no. 057
Plasminogen, code no. A081, lot no. 099
Albumin, code no. A001, lot no. 025
Hemoglobin, code no. A118, lot. no. 027
Alpha-1 antitrypsin, code no. A012, lot no. 018
Complement factor B (C factor B), code no. A343, lot. no. 016
Complement factor 5 (C5), code no. A055, lot no. 034
C1 esterase inhibitor, code no. A253, lot no. 013
Complement factor 3 split product c (C3c), code no. A062, lot no. 096
Complement factor 4 split product c (C4c), code no. A065, lot no. 015
Fibronectin, code no. A245, lot no. 117
Tetranectin, code no. A371, lot no. 096
Prothrombin, code no. A325, lot no. 015
GC-globulin, code no. A021, lot. no. 086
Alpha-2 plasmin inhibitor, code no. A303, lot no. 098
Beta lipoprotein, code no. A60, lot no. 025
Immunoglobulin A (alpha-chains), code no. A092, lot no. 018
Alpha-1 antichymotrypsin, code no. A022, lot no. 015
Hemopexin, code no. A064, lot no. 101

Rocket immunoelectrophoresis:
Rocket immunoelectrophoresis was performed on 11×20.5 cm glass plates in a 1 % agarose gel (Indubiose A 37, L'Industrie Biologique Francaise) with a thickness of 1.5 mm. The gels contained 4 % polyethylenglycol in order to enhance precipitation. TRIS-barbital buffer, pH 8.5 was used and the electrophoresis was run at 2.5 V/cm for 18 hours. The antibody preparations were used at a concentration of 1:100 (antibody preparation: gel, v/v) and wells with a diameter of 2.5 mm were punched in the gel. The distance between the wells was 5 mm and the wells received 5 µl samples of the various animal serum samples.

RESULTS

Table I shows the result of the reactions between animal proteins and antisera against human proteins. 2 indicates strong cross reaction, 1 indicates weak cross reaction and 0 indicates no cross reaction.

DISCUSSION

The present analysis demonstrates that antisera raised against human serum proteins in many cases can be used to study analogous proteins in animals.
The rocket immunoelectrophoretic assay employed has the advantage that it requires the presence of several antigenic determinants on the protein surface to react with the antibodies in order to form an immobile precipitate. This ensures that the cross reaction recorded is substantial and that other assays such as enzyme linked immunosorbent assays and radio immunoassays can also be applied to analyse the same antigen-antibody system. Another advantage of rocket immunoelectrophoresis is that it provides a qualitative result because the intensity of the precipitate resulting from the reaction with the animal protein compared with the human protein indicates the extent of immunological cross reaction between the human and the animal protein i.e. a week

Table I. Rocket immunoelectrophoretic analysis of serum from a number of animal species tested in assays employing different commercial antisera (Dakopatts) against human serum proteins.

	Monkey	Hen	Seal	Elephant	Musk ox	Wildebeest	Deer	Sheep	Goat	Horse	Pig	Cow	Dog	Cat	Raccoon dog	Polecat	Mink	Guinea pig	Hamster	Rat	Mouse
AFP		0	0	0	0	2	0		0	0	0	0	0	2			0	0	0	0	0
Albumin	2	2	2	2	2		2		2	2	2	2	2	2	2	2	2	2	2	2	2
α1-antichymotrypsin		0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
α1-antitrypsin	0	0	2	0	0	0	0		0	0	1	0	0	0	0	0	0	0	0	1	0
α2M	2	0	1	0	2		2	2	1	1	1	2	1	2	0	0	1	1	1	1	1
α2 plasmin inh.		0	1	0	0	0	0		1	0	0	0	0	0	0	0	0	1	1	0	0
β lipo protein		1	1	0	0	0	2		1	2	2	2	2	0	1	1	0	2	1	0	0
CBG		0		0	0	0	0		1	0	1	0	0		0	0	0	0	0	0	0
C3c								2	2	2	2	2	1	1			2	2		2	2
C4c								1	1	2	2	1	2	2			2	1		2	1
Ceruloplasmin	1	0	2	1	2		2	2	1	2	1	2	2	2			2	1	0	1	2
Cl esterase inh.	0	0	0	0	0	0	1		0	0	0	0	0	0	0	0	0	0	0	0	1
C factor B	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
C5	0	0	1	1	1	1	1		0	1	0	0	0	0	0	0	0	1	2	1	1
Fibronectin	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
GC-globulin		2	2	2	0	0	2		2	1	2	2	2	1	1	2	2	2	1	2	2
Haptoglobin	0	0	2	2	0	0	0	0	1	2	2	0	2	2	2	2	2	1	2	2	1
hCG		0		0	1	2	0		0	1	1	0	0		0	1	0	0	1	0	1
Hemoglobin (hemol. serum)		1	1	1	2	1	2		0	1	1	1	1	0	1	2	1	1	1	0	1
Hemopexin		0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1
hPL		0		0	2	2	0		0	0	0	0	0		0	0	0	0	0	0	0
IgA (α-chains)		0	0	0	0	0	1	1	1	1	1	1	0	0	0	2	1	0	0	0	1
IgG	2	0	0	0	1		1		1	1	1	1	1	2	1	1	1	0	0	1	1
IgM	0	0	2	1	0		1		0	2	1	1	2	1	2	2	2	0	1	1	1
Orosomucoid	0	0	0	0	0	0	0		0	0	0	0	0		0	0	0	0	0	0	0
PAPP-A		0		0	0	1	0		0	0	0	0	0		0	0	0	0	0	0	0
Prealbumin	0	0	0	0	0		0		0	0	1	0	1	1	1	1	0	0	0	0	0
Prothrombin		0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
PZP		0	0	1	2	1	0		0	0	0	0	0		0	0	0	0	0	0	0
SP1		0		0	0	2	0		0	0	0	0	0		0	0	0	0	0	0	0
Tetranectin		2	2	2	2	2	2	2	2	2	1	2	2	1	2	2	2	2	2	1	2
Transferrin	0	0	0	0	0		0		0	0	0	0	0	0	1	1	1	0	0	0	0

precipitate indicates a minor cross reactivity than a strong precipitate.

The many negative cross reaction results published in the present paper do not necessarily indicate that these proteins in the animals do not share common properties with the human analogue. It just means that the antisera employed in this study, which were all raised in rabbits, do not contain sufficient amounts of antibodies with specificities against common epitopes of the human and the animal protein to allow precipitate formation between the animal protein and the rabbit antiserum raised against the human analogue. A negative result may also in certain instances e.g. AFP indicate that the protein is present at a low serum concentration i.e. below the detection limit of rocket immunoelectrophoretic assays.

Demonstration of immunological cross reaction between an animal protein and a human analogue is often desirable when an animal species is to be used as a model for the study of the human protein. If the proteins do not share many antigenic determinants it may be necessary to raise antisera in

a phylogenetically remote species e.g. birds (*Hau et al.* 1981), and/or to apply more sophisticated assays e.g. line immunoelectrophoresis to the analysis (*Hau et al.* 1980). Application of other assays in the present study might have resulted in the demonstration of more extensive cross reaction, but we deliberately chose rocket immunoelectrophoresis as the test system in order to ensure that the results are applicable regardless of which assay might be chosen by others on the basis of a positive result published in this paper.

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Summary
In the present rocket immunoelectrophoretic analysis commercially available antisera against human serum proteins were screened for their usability in the analysis of analogous proteins in a number of animal species.
The result appears as a table which demonstrates to what extent antibodies raised against a human protein can be used in the quantitative and/or qualitative study of an analogous animal protein.

Sammendrag
Brugbarheden af antistoffer mod en lang række humane proteiner til analyse af analoge proteiner hos en række forskellige dyrearter blev undersøgt ved brug af præcipitationsassayet raket immunoelektroforese.
Resultatet af den immunologiske krydsreaktionsundersøgelse præsenteres som en tabel, hvor 2 indicerer kraftig krydsreaktion, 1 svag krydsreaktion og 0 ingen krydsreaktion.

Yhteenveto / K. Pelkonen
Tässa työssä selvitettiin kaupallisesti saatavien antiseerumien käyttökelpoisuutta useiden eläinlajien analogisten proteiinien analyysiin, käyttäen rocket immunoelektroforeettista analyysiä. Tulokset esitetääntaulokon muodossa, josta voi havaita kunkin ihmisproteiinin antibodin soveltuvuuden kvantitatiiviseen ja/tai kvalitatiiviseen analogisen eläinproteiinin määrittämiseen.

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