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Phylogenetic Heterogeneity of Plasma Kininogens* (40405)

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Kininogens are plasma proteins from which vasoactive polypeptides, such as bradykinin, are released by certain proteolytic enzymes. Recently, high molecular weight kininogens have been found to function in the blood clotting mechanism by enabling Hageman factor (factor XII) to promote surface-dependent reactions which lead to plasma clotting, release of kinin, and fibrinolysis (1–5). Since Hageman factor (factor XII) is present in mammalian plasmas except for cetaceans (6, 7) and is required for surface-induced clotting of plasma (8), we have examined the functional and antigenic properties of the plasma kininogens in nonhuman primates and other species. Saito and his colleagues described clotting activity due to high molecular weight kininogen in a number of mammalian plasmas, including that from cetaceans (9). The low molecular weight kininogens in human plasma lack clotting activity (2–4).

The following studies demonstrate disparities between the functional and antigenic properties of human high molecular weight kininogens in plasma from nonhuman species. In certain primates the clotting activity attributable to high molecular weight kininogen was present despite a marked deficiency of its antigenic properties. In other mammals, no kininogen antigens were detectable immunologically despite the fact that clotting activity of high molecular weight kininogen (Fitzgerald factor) (1) could be measured.

Materials and methods. Human plasma which was markedly deficient in all species of kininogen molecules, and that deficient in only high molecular weight kininogen, was obtained from individuals with inherited deficiencies of each, reported earlier (1, 4). Plasma was separated in this laboratory from blood which had been drawn with silicone coated (SC-87, G-E Dri-film, General Electric, Watertown, New York) syringes and test tubes containing 1/50 volume of 0.5M buffered citrate, pH 5.2 and rendered platelet deficient, as described earlier (4). Plasma samples and some serum samples from primates had been obtained through Dr. H. Moor-Jankowski from the Holloman Air Force Base primate colony. Other samples of plasma and serum from non-human species were obtained from animals housed in the animal quarters of The Children's Hospital Research Foundation, Cincinnati, Ohio, or from commercial sources, including the Animal Blood Center, Syracuse, New York. Cetacean plasmas were obtained from Dr. G. Fuller, Galveston, Texas. All samples of plasma and serum were stored at −30° or −70° until used. The nonhuman samples had been previously thawed.

Agarose to prepare 1% gels for immunodiffusion was obtained from l'Industrie Biologique Francaise, via Fisher Scientific, Cincinnati, Ohio. Kaolin (Fisher Scientific, Cincinnati, Ohio) and a phospholipid, Centrolex-O (Central Soya Products, Chicago, IL) were used in assays to measure clot-promoting activity. Human O, Rh negative erythrocytes were used to prepare cells for hemagglutination assays, used in quantifying high molecular weight kininogen as described earlier (10). The buffer used in clotting assays was prepared with sodium barbital (2.06 g/L), barbital (2.76 g/L), and sodium chloride (7.3 g/L), at pH 7.4.

Ouchterloney double agar diffusion was carried out at room temperature using 1% agarose gels prepared in pH 7.4 barbital buffer. Clotting assays to quantify Fitzgerald factor, or other clotting factor activities, were performed as reported earlier (1, 4, 8, 11). The clotting assay measured the capacity of dilutions of test plasma to shorten the clotting time of plasma from an individual with a hereditary deficiency of plasma kininogens (less than 1% of normal) (4, 12). The quantity of clot-promoting activity attributable to Fitz-

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gerald factor was estimated by comparing clotting times of dilutions of test plasma samples with those of identical dilutions of pooled normal human plasma which had been charted on a double logarithmic graph.

Antiserum to human plasma kininogens, described earlier (10, 12), reacted with antigenic determinants of both high and low molecular weight kininogens in double agar diffusion. Monospecific antibody to human high molecular weight kininogen was prepared from this antiserum by exhaustively adsorbing it with a preparation of low molecular weight kininogen. This antiserum to only high molecular weight kininogen did not give a precipitin reaction with high or low molecular weight kininogen or with human plasma in double agar diffusion, but would agglutinate erythrocytes coated with high molecular weight kininogen (10).

Results. When plasma from a number of species was reacted with a specific antiserum to both human high and low molecular weight kininogens (12) in 1% agarose gels, samples from non-human primates gave precipitin reactions, while those from other mammals, including cetaceans, and avia did not (Table I, Fig. 1). When the precipitin reactions between primate plasma samples were compared, the kininogen antigens of squirrel monkey plasma were deficient with respect to those of all other primates, including a human plasma deficient only in high molecular weight kininogen (1), as illustrated in Figure 1; A, B, E, F. Chimpanzee plasma samples gave reactions of complete immunological identity with pooled human plasma in most instances, but some chimpanzee plasmas (not shown) gave faint reactions of partial identity in which the direction of spurring indicated antigenic deficiency of the chimpanzee kininogen (Figure 1; D, E, J, and Table II). Some baboon plasma kininogen antigens were deficient with respect to both human and chimpanzee (Figure 1, C); others gave reactions of complete identity with the human and chimpanzee plasmas (Figure 1; A, B). The antigens in black ape plasma were immunologically identical to those in other normal primate plasmas but were deficient with respect to high molecular weight kininogen deficient plasma (Fig. 1, K). Plasma or serum samples from other mammals and birds gave no precipitin reactions with the antihuman kininogen serum (Table I, Fig. 1).

TABLE I. SPECIES DIFFERENCES IN KININOGENS[a]

Species	Antigens cross-reactive with human kininogen antigens (Precipitin reaction)	HMW – KGN: Antigen (HIA)	HMW – KGN: Clotting activity
NHP	+	+	+
Chimpanzee	+	+	+
Baboon	+	0	+
Gibbon	+	0	+
Black ape	+	0	+
Rhesus	+	0	+
Squirrel monkey	+	0	+
Horse	0	n.t.	+
Dog	0	0	+
Rabbit	0	n.t.	+
Guinea pig	0	0	+
Goat	0	0	+
Calf	0	0	0
Fetal calf	0	n.t.	0
Rat	0	n.t.	n.t.
Whale	0	0	+
Dolphin	0	0	+
Duck	0	0	0
Chick	0	n.t.	0

[a] Plasma samples from the species listed in the left column were tested for their content of antigens recognized by antibody against all species of human kininogens (12) in agarose double diffusion (second column). Some were tested for their content of antigens specific for human high molecular weight kininogen (HMW-KGN) in a hemagglutination inhibition assay (HIA, column 3) (10) and for HMW-KGN clotting activity (Fitzgerald factor activity, last column) (1, 4). Samples containing less than 1% of the normal HMW-KGN clotting activity or less than 2% of the antigenic properties are designated as 0; n.t. = not tested.

Despite these indications of heterogeneity of kininogen antigens in primate plasma samples, all contained Fitzgerald factor clotting activity. Gibbon and chimpanzee plasmas gave reactions of complete identity in double agar diffusion against the antiserum to human plasma kininogens, and gibbon plasma contained as much Fitzgerald factor clotting activity as the human, but the chimpanzee plasma contained about half as much (Table II). Conversely, plasma from the squirrel monkey, which was markedly deficient in kininogen antigens, contained the same amount of Fitzgerald factor clotting activity as the human. The level of high molecular weight kininogen antigens in plasma functionally deficient in this protein may reflect non-functional high molecular weight kini-

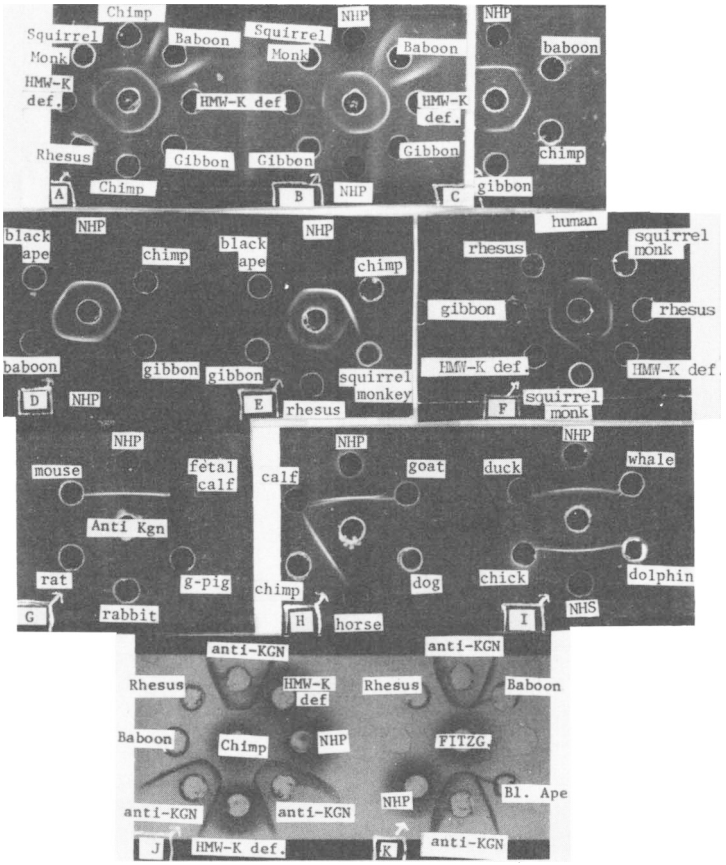

FIG. 1. Photographs of precipitin reactions between plasma or serum samples from various species and a monospecific antibody to human plasma kininogens which recognizes both high and low molecular weight kininogens (10). The center wells in Sets A through I contained the antikininogen serum. Sets J and K were stained with Coomassie brilliant blue before photography; the rest were wet gels. HMW-K def. plasma was from Mr. Fitzgerald. The baboon plasma (Sets A and B) contained antibody to unidentified antigens in plasma from other primates. The wells at 9 and 3 o'clock in Set K were not filled. NHP = normal human plasma; NHS = normal human serum.

nogen in this plasma (Table II, line 3).

When the antigenic properties of human high molecular weight kininogens in primate serum and plasma samples were compared, there was a dissociation between the concentration of antigenic determinants and the concentration of Fitzgerald factor clotting activity (Table II). Only chimpanzee and human plasmas contained these antigens. Although the gibbon, baboon, black ape, rhesus, and squirrel monkey plasma samples contained undetectable amounts of high molecular weight kininogen antigens, all contained significant amounts of Fitzgerald factor clotting activity. The range of Fitzgerald factor clotting activity was extremely wide in baboon plasma and serum samples. Some baboon plasmas gave precipitin reactions with other primate plasmas because certain baboons had been immunized with primate serum (Fig. 1, A and B) while others had not (Fig. 1 C). The antibody in the anti-*high* molecular weight kininogen serum gave no precipitin bands in this assay.

Discussion. These studies suggest dissociation in the evolution of the antigenic and clot-promoting properties of plasma kininogens. As Saito and his colleagues reported (9), the concentration of clot-promoting activity of high molecular weight kininogen (Fitzgerald factor) (1–4) varied widely among mammals, while avian, amphibian, and reptilian plasmas were markedly deficient in this property. The clot-promoting activity of bovine and

TABLE II. PLASMA KININOGENS (KGNs) IN PRIMATES.[a]

Species	Immunological reaction cf. normal human plasma	HMW-KGN μg/ml	Fitzgerald factor, % of normal human plasma
Human (pool)	Identical	90	100
KGN-def. human	0	0	0
HMW-KGN def. human	Identical	10	0
Chimpanzee	Identical and Deficient	50–60	54
Gibbon	Identical	<2	115
Baboon	Identical and Deficient	<2	44
Black Ape	Identical and Deficient	<2	75
Rhesus	Identical	<2	100
Squirrel monkey	Deficient	<2	100

[a] Plasma samples from the primates listed were tested in double agar diffusion with monospecific antiserum to human plasma kininogens for their immunologic resemblence to normal human plasma reacted with this antiserum. The high molecular weight kininogen (HMW-KGN) was quantified using a hemagglutination inhibition assay and a monospecific antiserum to human high molecular weight kininogen, described earlier (10), and Fitzgerald factor concentration determined in an assay which measures the clot-promoting activity of high molecular weight kininogen (4), and was performed on three different plasma samples from chimpanzees, baboons, gibbons, and two samples from black apes, rhesus and squirrel monkeys; mean values are given. Baboon sera which had been stored for a prolonged period contained only 1–25% of the concentration in normal plasma; normal human plasma contained the same concentration of HMW-KGN as normal human serum. The KGN-def. human plasma was from a subject reported earlier (4, 12) and HMW-KGN-def. plasma from Mr. Fitzgerald (1). A pool of 10 normal human plasma was used.

human high molecular weight kininogens is restricted to the light chain of 2-chain molecules (13, 14, 15), but human high molecular weight kininogen appears to exist in plasma as a single chain molecule (13). The antigenic parts of the kininogen molecules must be distant from this site.

Others have reported that bovine high molecular weight kininogen can partially correct the coagulation defect in human plasma deficient in high molecular weight kininogen (16), but calf and fetal calf plasma samples tested in the present studies lacked this activity (Table I). It is likely that the kinin releasing mechanism in these plasmas, which were obtained commercially, was activated during storage and shipment of the plasma in glass vessels, and that kinin was released. The bovine high molecular weight kininogen differs from the human in that it loses its clot promoting activity when bradykinin has been released (16–19). It is also possible that a plasma proteolytic enzyme, such as plasmin, may have destroyed the clot-promoting properties of this kininogen during storage of the plasma (20).

The antigenic properties of kininogen molecules in human plasma were also present in primate plasmas but not in the other plasmas tested even though they contained Fitzgerald factor clotting activity (Table I). Therefore, the clot-promoting portion of the high molecular weight kininogen molecules must differ from their antigenic portions. In earlier studies from this laboratory we found similar evolutionary differences in the functional and antigenic properties of another alpha globulin in plasma (serum inhibitor of an esterase derived from the first component of complement, $C\bar{1}$-INH) (21), but rhesus monkey inhibitor antigens were deficient with respect to those of human, chimp, and gibbon.

Several primate plasma samples contained no high molecular weight kininogen antigens detectable by hemagglutination inhibition but gave precipitin reactions with specific antiserum to both human plasma kininogens; the antibody to the high molecular weight kininogen does not give a precipitin reaction in double agar diffusion (10). The reason for this has not been determined, but it might be due to the reaction of a single antibody binding site with an antigenic determinant of the kininogen so that an aggregate large enough to form a precipitin reaction cannot be formed.

Summary. Plasma kininogens of a number of mammalian species differed from one another with respect to their antigenic properties detected with antiserum to human plasma kininogens. Precipitating antigenic properties of human plasma kininogens were found only

in primates, and nonprecipitating antigens resembling human high molecular weight kininogen only in human and chimpanzee plasmas. Even so, the clot-promoting activity of high molecular weight kininogen was widely distributed among primates and other mammals.

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