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INTERACTION OF GIBBON ANTIBODY WITH PRIMATE AND NON-PRIMATE COMPLEMENTS

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Summary

Gibbon anti-DNP fixed monkey, gibbon and human complements more efficiently than rat, guinea pig and dog complements. Rabbit antibody of similar specificity and affinity on the other hand fixed non-primate complements more efficiently than primate complements. Both antibodies were indistinguishable from one another with regard to the ability to fix rabbit complement.

Introduction

Information regarding the ability of primate antibodies to activate complement from different species of animals is not as readily available as that of non-primate antibodies, e.g., mouse, rat, rabbit, guinea pig and birds[1,2]. While these non-primate antibodies are capable of activating complements from many species of animals, little is known of their ability to activate primate complements[2,3]. Human antibody has been reported to fix rabbit complement more effectively than the more commonly used guinea pig complement[3]. Our limited recent observation suggested that purified gibbon anti-DNP fixed rabbit complement better than guinea pig complement[4]. The purpose of the present study is to extend this investigation further by analyzing the ability of the gibbon antibody to activate other primate and non-primate complements. Gibbon antibody was selected to represent the primate antibody on the basis that this primate is phylogenetically closest to man that can be readily handled in the laboratory.

Materials and Methods

2, 4 dinitrophenylated bovine gamma globulin (DNP-BGG) containing 50 moles of DNP/mole of BGG and 2, 4 dinitrophenylated human serum albumin (DNP-HSA) containing 24 moles of DNP/mole of HSA were prepared as described by Eisen and Siskind[5]. Gibbons (*Hylobates lar)* were hyperimmunized with DNP-BGG and bled as described[4]. Rabbits were immunized with 2 mg of DNP-BGG in complete Freund's adjuvant into the 4 footpads and bled 3 weeks later. Antibodies specific for DNP

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determinant were purified from pooled sera by an immunoadsorbent technique using DNP-L-lysine Sepharose column, and the antibodies were eluted with 10% dioxane in 0.1 M acetic acid[4]. Antibody prepared by this method was found to contain only one protein component by immunoelectrophoresis using appropriate polyvalent antisera and was identified as IgG by their reactivity with monospecific anti-IgG sera[4,6]. Protein concentration was determined spectrophotometrically[4]. The average intrinsic association constant (K_o) of purified gibbon and rabbit anti-DNP were 1.24×10^6 and 4.32×10^6 M^{-1} respectively[4]. Fresh sera used as a source of complement were pooled from several normal humans, gibbons, monkeys, dogs, rabbits and rats and the sera kept at -70°C until used. Lyophilized guinea pig complement was obtained from Travenal Laboratories, Costa Mesa, Calif., U.S.A. All sera were appropriately absorbed with sheep red blood cells (SRBC) snd were titrated for complement activity using SRBC sensitized with rabbit hemolysin[7].

The complement fixation method used was slightly modified from that described by Levine[8], employing both primate (human, gibbon and monkey) and non-primate (dog, rabbit, rat and guinea pig) complements. One ml of antibody solution containing 40 μg of purified anti-DNP and a volume of serum diluted to contain 2 CH_{50} units of complement were added to a series of tubes containing different quantities of DNP-HSA, and the total volume was made up to 6 ml with complement diluent. The reaction mixture was incubated at 4°C for 16-18 h before 1 ml of 0.25% SRBC suspension optimally sensitized with rabbit hemolysin was added. Hemolysis was allowed to proceed at 37°C for 60 min and the reaction was terminated by immersing the tube in an ice-bath and unhemolysed cells were immediately removed by centrifugation at 4°C. The degree of hemolysis was determined spectrophotometrically at 413 nm and expressed as per cent of the complement fixed.

Results and Discussion

The complement-fixing capacity of gibbon IgG antibody was markedly different from that of rabbit antibody of the same specificity and similar antigen-binding capacity (Fig. 1). In general, gibbon antibody fixes primate complements (Fig. 1A) more efficiently than it does non-primate except rabbit complements (Fig. 1 B). The opposite results were obtained when rabbit antibody was similarly analyzed (Figs 1C and 1D). As shown in the figure, gibbon antibody interacted equally well with the three primate complements (human, gibbon and monkey). Under the conditions used, more than 40% of the primate complements in the test system was fixed by gibbon antibody, but only about 10% of these complements was fixed by rabbit antibody. The similarity in the capacity of these three primate complements to interact with either gibbon antibody or rabbit antibody is not unexpected as Schur *et al.*[9] recently reported that components of complement from several primates were antigenically similar to one another.

The observation that gibbon antibody fixed guinea pig complement poorly comparing with rabbit complement is consistent with the previous observation of Hoet and associates who used human antibody in their study[3]. Antibodies from other

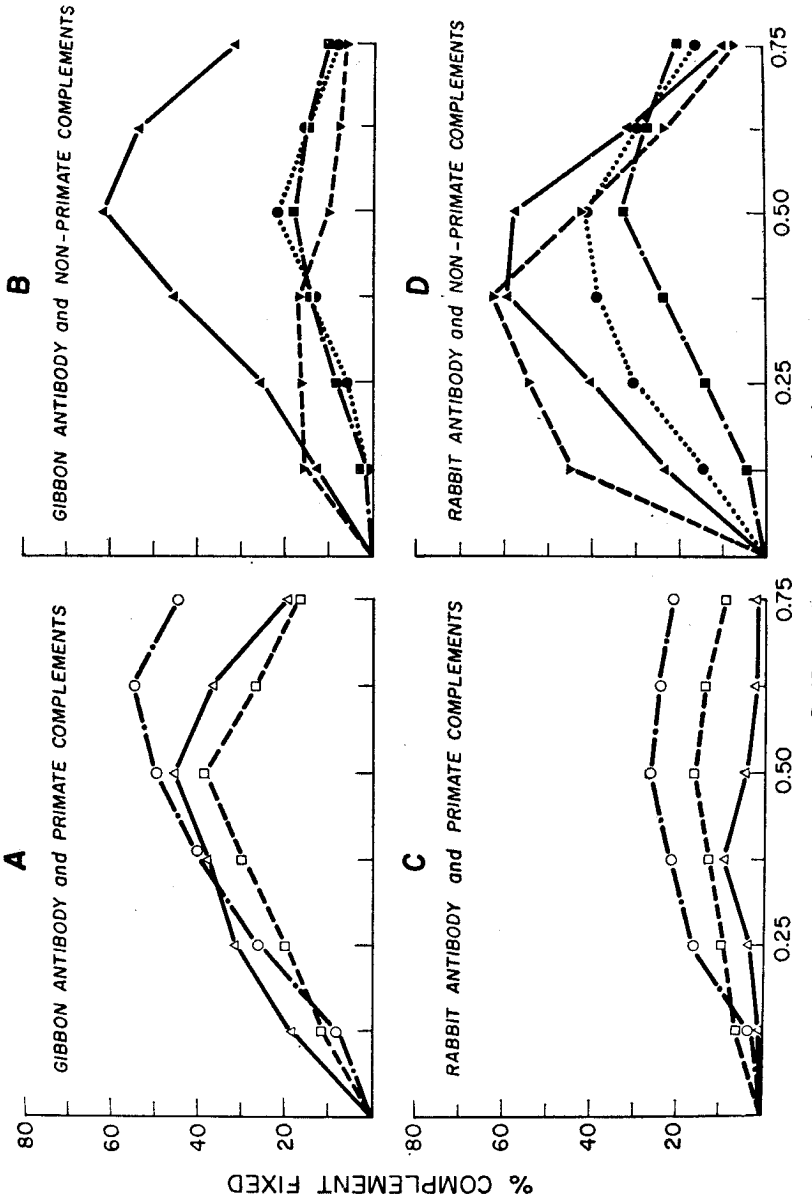

Fig. 1. Complement fixation curves showing the difference between gibbon antibody (K_0 = 1.24 × 10^6 M^{-1}) and rabbit antibody (K_0 = 4.32 × 10^6 M^{-1}) in the ability to fix primate (monkey O—·—O, gibbon □------□, and human △——△) and non-primate (rat ●·····●, guinea pig ▼-------▼, dog ■—·—■, and rabbit ▲——▲) complements. The quantity of antibody employed in each titration was 40 *μg*.

species of primates should be tested before one concludes that such an observation is a general characteristics of the primate antibodies. In addition, the use of erythrocytes and hemolysin from other species or alterations of the various parameters used in the test system should also be investigated in order to obtain a more optimal experimental condition for these antibodies and complements. Results from the present data nevertheless suggest that primate complements should be used preferentially during the study of primate antibodies. However, if these complements are not readily available, rabbit complement but not guinea pig can be used as a substitution for the primate complements.

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บทคัดย่อ

แอนติบอดีของชะนีที่ถูกสร้างขึ้นมาโดยการกระตุ้นด้วยสาร DNP มีความสามารถในการจับ (fix) กับคอมพลีเม้นท์ (complement) ของลิง (monkey) ของชะนี (gibbon) หรือของคน (human) ได้อย่างมีประสิทธิภาพดีกว่าคอมพลีเม้นท์ของหนู (rat) ของหนูตะเภา (guinea pig) หรือของสุนัข (dog) แต่แอนติบอดีของกระต่าย (rabbit) ที่มี specificity และ affinity เหมือนกับแอนติบอดีของชะนีนั้นให้ผลในการจับ (fix) กับคอมพลีเม้นท์ของสัตว์เหล่านี้ตรงกันข้าม คือมันจับกับคอมพลีเม้นท์ของพวก non-primate ได้ดีกว่าของพวก primate อย่างไรก็ตาม แอนติบอดีทั้งของกระต่ายหรือชะนีมีความสามารถในการจับกับคอมพลีเม้นท์ของกระต่ายได้ไม่แตกต่างกัน