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Sensitive Enzyme-linked Immunosorbent Assay (ELISA) Method to Measure Parasite-specific Antibodies of Mongolian Gerbils

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Abstract

Immunoglobulin G (IgG) of Mongolian gerbil, *Meriones unguiculatus*, was purified by an immunoaffinity chromatography and antibodies against it was raised in rabbits. Using this rabbit anti-gerbil IgG antibody, sensitivity of ELISA to measure parasite-specific antibodies in the sera of Mongolian gerbils was markedly enhanced than the previous method using rabbit anti-mouse IgG antibody, which could cross-react to gerbil IgG. Since Mongolian gerbils could effectively produce circulating antibodies against two intestinal helminths, *Strongyloides ratti* and *Nippostrongylus brasiliensis*, the high susceptibility of this animal to an array of parasites seems to be not due to general immunological deficiency.

Key words: Mongolian gerbils, *Meriones unguiculatus*, IgG, antibody, ELISA

Introduction

As an experimental animal, Mongolian gerbil, *Meriones unguiculatus*, is not as popular as mice or rats and has been mainly used as a model for studying epilepsy (Kaplan & Miezejeski, 1972) or cerebral infarction (Levine and Payan, 1966; Kahn, 1972). Recently we have found that Mongolian gerbils could not expel *Strongyloides* spp., though they could normally expel *Nippostrongylus brasiliensis* (Horii and Nawa, 1992, Horii *et al.*, 1993). This selective defect in the mucosal defence seems to be due to, at least in part, peculiar phenotype of mucosal mast cells of this animal (Horii *et al.*, 1992; Nawa *et al.*, 1994). Not merely to *Strongyloides* spp., Mongolian gerbils show high susceptibility to an array of infectious agents (Rich, 1968) including several helminth parasites (Ash and Riley, 1970, Cross *et al.*, 1978; Panitz and Shum, 1981). Since high susceptibility of Mongolian gerbils to such a wide range of pathogens cannot be explained simply by the uniqueness of mast cell phenotypes, general defence system of this animal should have certain differences from that of other experimental animals such as mice and rats. Unfortunately, however, hardly any basic information of the immune system of Mongolian gerbil has been available until now. Even simple antibody titration, western blotting or other immunological measurements has been done using each research groups' own rabbit anti-gerbil IgG antibody/antiserum (Yates and Higashi, 1985; Kazura *et al.*, 1986; Prier and Lammie, 1988; McVay *et al.*, 1990) or using commercially sold antiserum against mouse Igs (Lucius *et al.*, 1986, Khan *et al.*, 1993) or hamster IgG (Klei *et al.*, 1988). Therefore, production and distribution of appropriate tools such as antibodies against Ig subclasses and lymphocyte markers is essential to characterize immune responses of Mongolian gerbils against various parasites. To this end, we have purified IgG of Mongolian gerbils and raised rabbit anti-gerbil-IgG antibody. This conventional antibody gave satisfying results in ELISA to measure anti-parasite antibody responses of Mongolian gerbils.

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Materials and Methods

Animals

Inbred male Mongolian gerbils, *M. unguiculatus*, (MGS/Sea) maintained by sib mating over F_{40} were purchased from Seiwa Experimental Animals Ltd., (Yoshitomi, Fukuoka, Japan). The gerbils used in this study were males and 8 weeks old at the start of experiments. Female New Zealand White rabbits of 1–2 kg were purchased from Kuroda Experimental Animals Ltd., (Kumamoto, Japan). The gerbils and rabbits were kept under clean conventional conditions in the Experimental Animal Center, Miyazaki Medical College. Normal sera were obtained from mice (C57BL/6), rats (Wistar), and several other rodent species, *Mus platythrix, Phodopus campbelli, Cricetulus griseus, Cricetulus triton*, which were maintained in the Experimental Animal Center, Miyazaki Medical College.

Serial serum samples of Mongolian gerbils after parasite infections

Serum samples were obtained from Mongolian gerbils various time after infection with either *S. ratti* or *N. brasiliensis*. The animals were the same as those reported in our previous papers (for *S. ratti*, Horii and Nawa, 1992; for *N. brasiliensis*, Horii *et al.*, 1992).

Purification of gerbil immunoglobulin G (IgG)

Mongolian gerbils were immunized subcutaneously with 10 μg or 1 mg of bovine serum albumin (BSA; #A7030, Sigma, St. Louis, USA) emulsified in complete Freund's adjuvant (CFA; DIFCO, Detroit, MI, USA) followed by bi-weekly immunizations with the same amount of BSA emulsified in incomplete Freund's adjuvant (IFA: Nacalaitesc, Tokyo, Japan). Sera were obtained from the animals before and after the immunization and tested for the binding to BSA in ELISA as described below. Gerbil IgG was purified as anti-BSA antibodies by immunoaffinity chromatography. BSA was coupled to CNBr-activated Sepharose-4B (Pharmacia, Uppsala, Sweden) by standard protocol. Pooled sera of BSA-immunized gerbils were diluted in Tris-buffered saline (TBS) and applied on the column. After extensive washing with TBS, gerbil anti-BSA IgG was eluted with 0.5 M glycine HCl buffer, pH2.3. The eluates were neutralized with Tris base and dialyzed against phosphate-buffered saline (PBS) at 4°C overnight. Purity of the gerbil IgG was checked in 15% polyacrylamide gel electrophoresis (PAGE) under reducing condition.

Preparation of rabbit anti-gerbil IgG

Rabbits were immunized with 200 μg of purified gerbil IgG in CFA, followed by two immunizations of 100 μg of gerbil IgG in IFA. Sera were collected before and after the immunization and tested for the reactivity and specificity to gerbil IgG by various ways as described in the Results section. In brief, immunoreactivity of the rabbit antisera against gerbil and other rodent immunoglobulins was tested by Ouchterlony's method and the specificity against gerbil IgG by immunoelectrophoresis.

Enzyme-linked immunosorbent assay (ELISA)

Immunoreactivity of commercially purchased anti-mouse IgGs against various rodent immunoglobulins was measured by sandwich ELISA. Wells of microtiter plates (#25805-96 Corning, N.Y., USA) were coated with 5 μg/ml of rabbit anti-mouse IgGs (Z 109, DAKO, A/S, Glostrup, Denmark) and blocked with TBS containing 1% casein. Various concentrations of sera from a panel of animals were added to the wells and the binding of rodent immunoglobulins was detected by horse radish peroxidase (HPO)-labelled goat anti-mouse IgG $F(ab')_2$ (1:2000 dilution, #55553, Cappel, Durham, NC, USA) followed by H_2O_2 as substrate and 2,2-azino-bis-(3-ethylbenzthiazoline-6 sulphonic acid) (ABTS) as color indicator. Optical densities (OD) were measured 1 hour later at 405 nm in a ELISA reader (Multiskan® Bichromatic, Labsystems Inc., Helsinki, Finland).

Binding of gerbil sera to BSA or parasite antigens was also measured in ELISA. Wells of microtiter plates (#25805-96 Corning) were coated with BSA (1 μg/ml) or parasite antigens (10 μg/ml) in carbonate buffer, pH9.6 and blocked with TBS containing 1% casein. Various concentrations of gerbil sera were added to the wells and the binding of gerbil IgG to the antigens was detected either by HPO-labelled goat anti-mouse IgG $F(ab')_2$ (1:2000 dilution, #55553, Cappel) or rabbit anti-gerbil IgG (1:1000 dilution) followed by HPO-labelled swine anti-rab-

bit IgG (1:1000 dilution, P 217, DAKO). Coloring and OD measurement were the same as those described above.

Results

Cross reactivity among rodent immunoglobulins

In order to evaluate the binding reactivities of commercially purchased anti-mouse IgGs to gerbil and other rodent immunoglobulins, wells of microtiter plates were coated with rabbit anti-mouse IgGs and then serially diluted normal sera of various rodents and human were added to each well. The immunoglobulins captured by anti-mouse IgGs were detected by HPO-labelled goat anti-mouse IgG $F(ab')_2$. The results were summarized in Fig. 1. Except the serum of *M. platythrix*, which belongs to the genus *Mus*, most rodent sera tested bound to anti-mouse IgGs less than half of that of mice. The binding of normal gerbil serum was less than 1/3 of that of normal mouse serum. Practically no binding was seen in human serum.

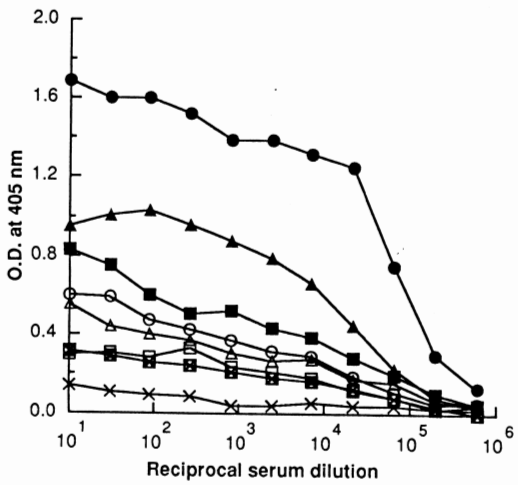


Fig. 1 Binding of rabbit anti-mouse IgG to various rodent sera.
Wells of microtiter plates were coated with rabbit anti-mouse IgG. After incubation with serially diluted sera from various rodents, the bound IgG was detected by HPO-labelled goat anti-mouse-$F(ab')_2$.
● : *Mus musculus*, ▲ : *Mus platythrix*, ■ : *Rattus norvegicus*, ○ : *Phodopus campbelli*, △ : *Meriones unguiculatus*, □: *Cricetulus griseus*, ⊠: *Cricetulus triton*, +: *Homo sapiens*.

Purification of gerbil IgG

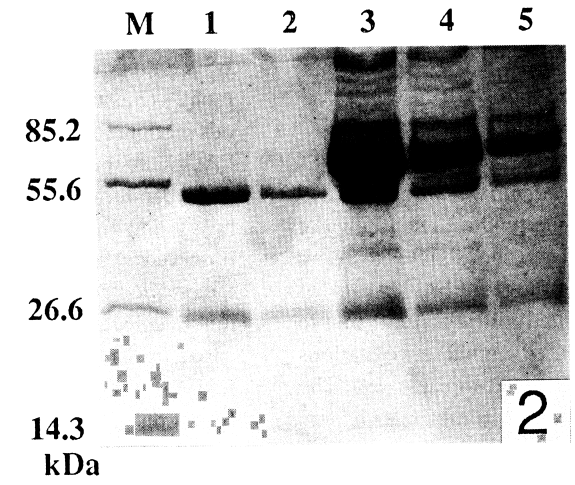
IgG of Mongolian gerbils was purified as anti-BSA antibodies using immunoaffinity chromatography as mentioned in detail in Materials and Methods section. Purity of the gerbil IgG was checked by PAGE under reducing condition (Fig. 2). Only two sharp bands of 55 kDa and 25 kDa corresponding to heavy and light chains of immunoglobulin were observed. After having raised rabbit antibodies to the purified gerbil IgG, purity was also checked by immunoelectrophoresis. The rabbit anti-gerbil IgG antiserum produced sharp single arch against purified gerbil IgG, while it could recognize at least two IgGs present in the normal gerbil serum (Fig. 3). Mouse anti-normal gerbil serum did not produce precipitin bands against gerbil IgGs or albumin, though this antisera produced clear precipitin bands against α- and β-globulins (Fig. 3). Since binding of this antisera to purified gerbil IgG was, though it was weak, detectable by ELISA, this weak reaction was probably due to the shared epitopes of these proteins of gerbils and mice.

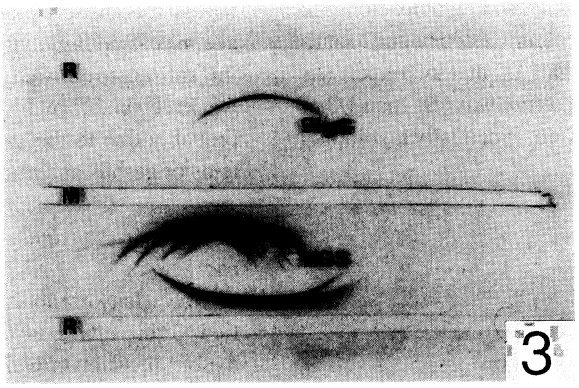
Binding of rabbit anti-gerbil IgG antisera to various rodent sera

Specificity of rabbit antisera raised against purified gerbil IgG was tested by an Ouchterlony's double diffusion in agar. Strong precipitin band was produced against 50-fold diluted normal gerbil serum, whereas far weaker bands were observed against undiluted sera of other rodents. No bands could be seen against human serum (Fig. 4).

Sensitivity of the ELISA using rabbit anti-gerbil IgG

To demonstrate the advantage of the use of rabbit anti-gerbil IgG in measuring gerbil antibodies, sera from BSA-immunized gerbils were tested for the binding to BSA in two different ELISA methods; in one system HPO-labelled goat anti-mouse IgG $F(ab')_2$ was used for detection of gerbil IgG while in the other system a combination of rabbit anti-gerbil IgG and HPO-labelled swine anti-rabbit IgG was used. As shown in Fig. 5, HPO-labelled goat anti-mouse IgG $F(ab')_2$ could detect binding of gerbil IgG to BSA only at lower dilutions of the sera and the maximum OD values were less than 0.4. On the other hand, rabbit anti-gerbil IgG followed by HPO-labelled swine anti-rabbit IgG could detect binding





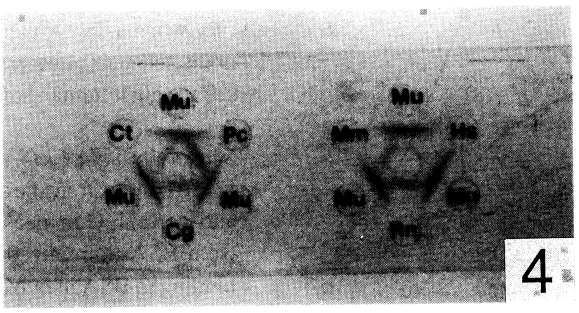


Fig. 2 SDS-PAGE of purified gerbil IgG and normal gerbil serum.
Lanes 1 and 2: purified gerbil IgG (3.2 μg for lane 1, 0.8 μg for lane 2), Lanes 3–5: normal gerbil serum (2.0 μl, 0.6 μl and 0.2 μl from lane 3 to lane 5), M: Molecular markers.

Fig. 3 Immunoelectrophoretic pattern of purified gerbil IgG.
R: rabbit anti-gerbil IgG, M: mouse anti-normal gerbil serum, gIgG: purified gerbil IgG, NGS: normal gerbil serum.

Fig. 4 Specificity of rabbit antiserum against gerbil-IgG by an Ouchterlony's method.
Center well: undiluted rabbit anti-gerbil IgG antiserum, Mu: *M.unguiculatus*, Ct: *C. triton*, Cg: *C. griseus*, Pc: *P. campbelli*, Mm: *M. musculus*, Rn: *R. norvegicus*, Hs: *H. sapiens*.
Normal gerbil serum (Mu) was diluted to 1:50, whereas sera from other animals were undiluted.

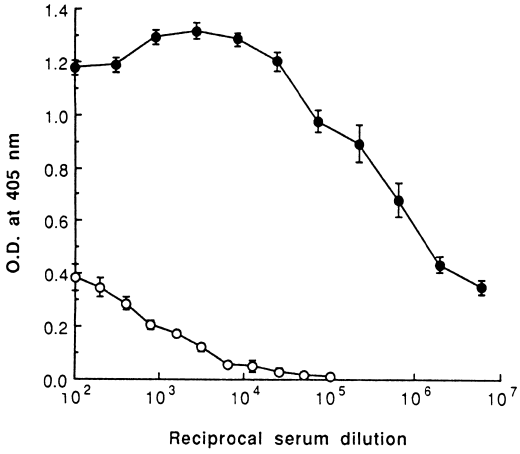


Fig. 5 Titration of gerbil antibody against BSA by ELISA. ●: detected by rabbit anti-gerbil IgG followed by HPO-labelled swine anti-rabbit IgG, ○: detected by HPO-labelled rabbit anti-mouse $F(ab')_2$.

of gerbil IgG to BSA at much higher dilutions all the way up to 10^{-7} and the maximum OD values were about 1.4.

Humoral immune responses of Mongolian gerbils to parasite antigens

To confirm further the advantage of the use of rabbit anti-gerbil IgG in measuring gerbil antibodies, sera from Mongolian gerbils infected with *S. ratti* or *N. brasiliensis* were tested for the binding to the corresponding parasite antigens in two different ELISA methods as described above. As shown in Figs. 6 and 7, regardless of the parasite used for infection, parasite-specific antibodies became detectable in the sera of infected gerbils as early as 1 week post-infection when a combination of rabbit anti-gerbil IgG followed by HPO-labelled swine anti-rabbit IgG was employed. The detection of parasite-specific gerbil antibodies by HPO-labelled rabbit anti-mouse IgG $F(ab')_2$ was far less sensitive than the other method.

Discussion

The present results clearly demonstrate that the sensitivity of the detection of parasite specific antibodies in the sera of Mongolian gerbils by the ELISA using rabbit anti-gerbil IgG was 5–7 times higher than that using rabbit-anti mouse IgG. Although such results were predictable, appropriate antiserum raised against gerbil IgG has never been sold commercially, in spite of the wide usage of Mongolian gerbils as an experimental host for various parasites. Nor has been much efforts paid for the improvement of the immunological assay systems. As mentioned in Introduction, most researchers had

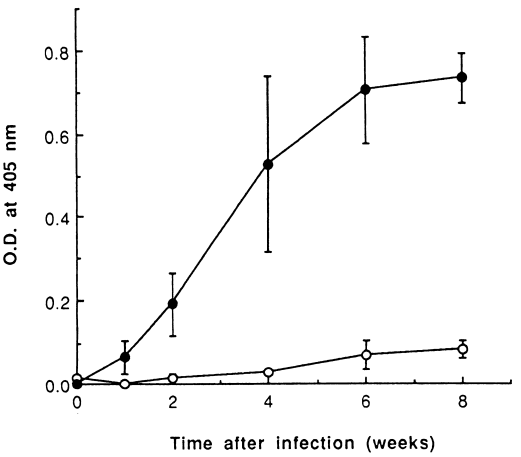


Fig. 6

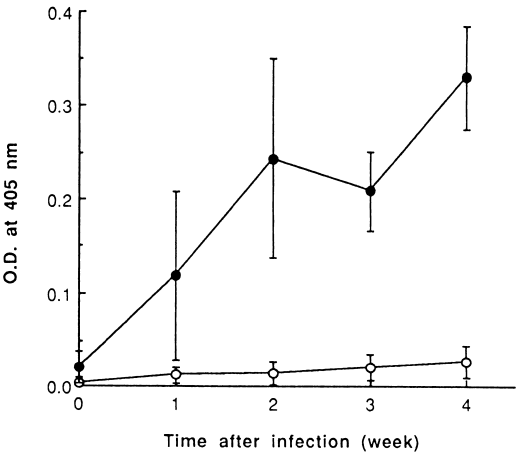


Fig. 7

Figs. 6, 7 Detection of parasite specific antibodies in the sera of gerbils infected with *S. ratti* (Fig. 6) or with *N. brasiliensis* (Fig. 7).
Symbols are the same as Fig. 5. All sera tested were diluted at 1:100.

used their own rabbit anti-gerbil IgG antibody to measure parasite-specific antibodies of gerbils and a few groups used commercially sold rabbit anti-mouse or anti-hamster IgG.

As the recommended methods for purification of immunoglobulins, combination of ion exchange chromatography on DEAE-cellulose and gel filtration on Sephadex G-200 has widely been used in many laboratories (Fahey and Terry, 1978). More recently the usage of protein-A or -G affinity chromatography was recommended for the purification of IgG (Bjorck and Kronvall, 1984). The classical column work is rather time-consuming and needs relatively large amount of serum as a starting material. The modern protein-A or -G affinity chromatography is relatively costly and its applicability to other animal IgGs than human and mouse IgG has not yet been established. Compare to these, immunoaffinity method described here is simple and less expensive and can theoretically be applicable to any animal species. In fact, we could successfully prepare rabbit anti-gerbil IgG within 2 months using only two gerbils immunized with BSA. Since we have made a bulk of rabbit anti-gerbil IgG antisera, portions of this could be distributed upon request with no obligation.

In the present study, we have found that Mongolian gerbils could produce circulating antibodies against two species of intestinal helminths as early as one week after a primary infection. The antibody titers rose up further with time. These results suggest, though not completely rule out, that the high susceptibility of Mongolian gerbils to various parasites is not due to defective antibody production. Application of the sensitive ELISA methods to an array of parasite infections in Mongolian gerbils would solidify this possibility.

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