Leucocytozoon caulleryi に対する鶏の免疫グロブリンMとGの抗体応答

誌名	Japanese journal of veterinary science
ISSN	00215295
著者名	磯部,尚 鈴木,恭
発行元	Japanese Society of Veterinary Science
巻/号	49巻2号
掲載ページ	p. 333-339
発行年月	1987年4月

Immunoglobulin M and G Immune Response to *Leucocytozoon caulleryi* in Chickens

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(Received 4 August 1986/Accepted 10 January 1987)

ABSTRACT. Immunoglobulin M and G immune response to *Leucocytozoon caulleryi* in chickens were investigated with the enzyme-linked immunosorbent assay. In chickens experimentally infected with *L. caulleryi*, IgM antibodies against schizonts were detected from 10 days after sporozoite inoculation and peaked at 3 weeks following inoculation. Thereafter they decreased and remained at a low level. However, IgG antibodies against schizonts were detected from 14 days after sporozoite inoculation, increased gradually, and reached a high level from 8 to 10 weeks after inoculation. On the other hand, in naturally infected chickens, IgM and IgG antibodies against schizonts were detected with/after detection of protozoa in blood smears. IgM antibodies subsequently decreased and maintained a lower level than that of IgG, although IgG antibodies against schizonts of *L. caulleryi* persisted at a high level for nearly one year. —KEY WORDS: chicken, IgG, IgM, immune response, *Leucocytozoon caulleryi*.

Jpn. J. Vet. Sci. 49(2): 333–339, 1987

Leucocytozoon caulleryi, an agent causing chicken leucocytozoonosis, was first described by Mathis and Leger [8] and Akiba *et al.* [1]. Leucocytozoonosis caused by infection with this parasite affects the productivity of chickens through a reduction in egg production, weight loss and sometimes death in various Asian countries. To date, antibody to *L. caulleryi* has been detected by the agar gel precipitation (AGP) test [12], the counterimmunoelectrophoresis [2] and the indirect immunofluorescent antibody test [5]. Recently, we reported that an enzyme-linked immunosorbent assay (ELISA) can be used as a highly sensitive and specific method for serodiagnosis of *L. caulleryi* infection in chickens [7]. However, only IgG class antibody response to *L. caulleryi* in chickens was measured in these reports. The present study deals with the immunoglobulin M immune response to *L. caulleryi* in chickens compared with that of IgG class using ELISA.

MATERIALS AND METHODS

Protozoa: The Gifu strain of *L. caulleryi* was used. It was isolated by the authors from naturally infected chickens at Seki, Gifu Prefecture, Japan, in July, of 1982, and has been maintained at the authors' laboratory by transmission in specific-pathogen-free (SPF) chickens and colonized *Culicoides arakawae*. The procedures of rearing, breeding and feeding of *C. arakawae* for infection with *L. caulleryi*, and preparation of sporozoite suspension for inoculation to chickens were the same as described previously [6, 10].

Chickens: SPF chickens of the PDL-1 strain were used. They were maintained at the authors' laboratory in the same manner as described by Furuta *et al.* [3].

Conjugate: Lyophilised anti-chicken immunoglobulin G, Fc fragment specific (Fc, prepared in goat), was obtained from Nordic Immunological Laboratories (Tilburg, The Netherlands) and anti-chicken immunoglobulin M, μ-chain specific (μ, pre-

pared in goat), was obtained from Cappel Laboratories (Pennsylvania, U.S.A.). After rehydration, these anti-chicken immunoglobulins were precipitated with one-third saturation of ammonium sulfate three times and labelled with horseradish peroxidase (Type VI, Sigma) by the method of Wilson and Nakane [17]. We used the anti-chicken immunoglobulin G, heavy and light chain specific (H & L, prepared in rabbits), conjugated with horseradish peroxidase. It was obtained from Cappel Laboratories (Pennsylvania, U.S.A.). These three conjugates were diluted with phosphate buffered saline (PBS, pH 7.2) containing 1% bovine serum albumin (Fraction V, Sigma) and stored in aliquots at −80°C until use. The optimal concentration of the IgM (μ) conjugate was 1: 40, that of IgG (Fc) conjugate was 1: 200, and that of IgG (H & L) conjugae was 1: 800, when determined by checkerboard titration. The negative cutoff point was set at 0.30 for IgM (μ) conjugate, at 0.10 for IgG (Fc) conjugate, and at 0.20 for IgG (H & L) conjugate, when determined using 0- to 40-week-old SPF chicken sera.

Antigens: Schizont antigen prepared from second generation schizonts were employed. The preparation of antigen was described in a previous report [7]. The protein concentration of the antigens were 6 μg/well for IgG (H & L) conjugate, 0.75 μg/well for IgM (μ) conjugate and 3 μg/well for IgG (Fc) conjugate, when determined by checkerboard titration.

Sera: Sera were collected from the following groups. i) Eight 93-day-old chickens were inoculated with 30 sporozoites of *L. caulleryi* intravenously. The serum samples were collected from them before, and every two days for 30 days after sporozoite inoculation. ii) Ten 28-day-old chickens were inoculated with 1×10^2 sporozoites of *L. caulleryi* intravenously. The serum samples were collected from them 1 week before, and every week for 10 weeks after sporozoite inoculation. iii) Ten 40-week-old chickens were kept in the chicken house and exposed to natural infection with *L. caul-*

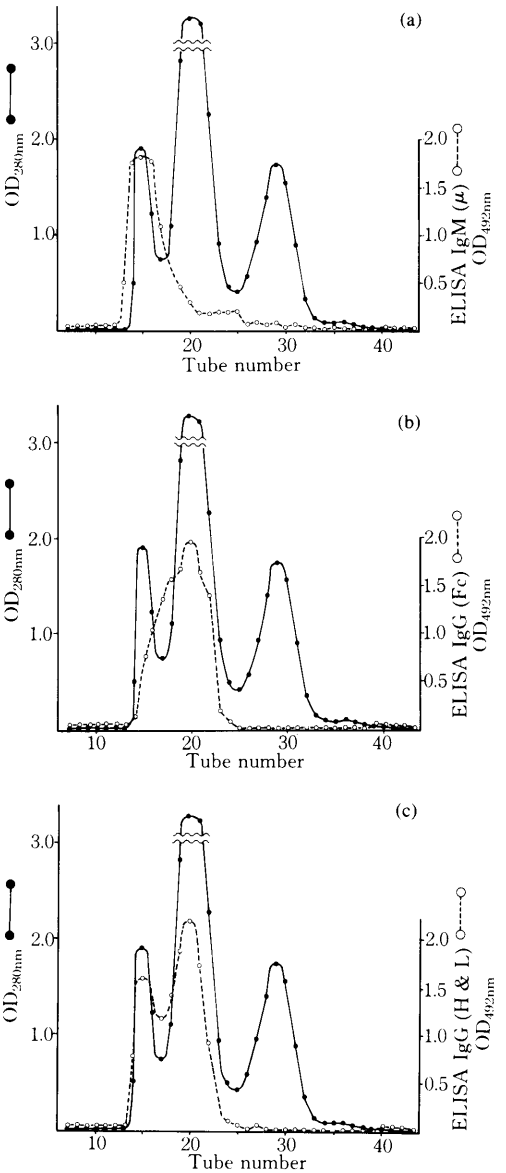

Fig. 1. Demonstration of antibodies to *L. caulleryi* in fractions after gel filtration of representative sera. (a) anti-chicken IgM (μ) conjugate; (b) anti-chicken IgG (Fc) conjugate; (c) anti-chicken IgG (H & L) conjugate; Column, Sephadex G-200 (2.6×32 cm); fraction volume, 3.0 ml/tube; flow rate, 7.0 ml/hr; OD, optical density.

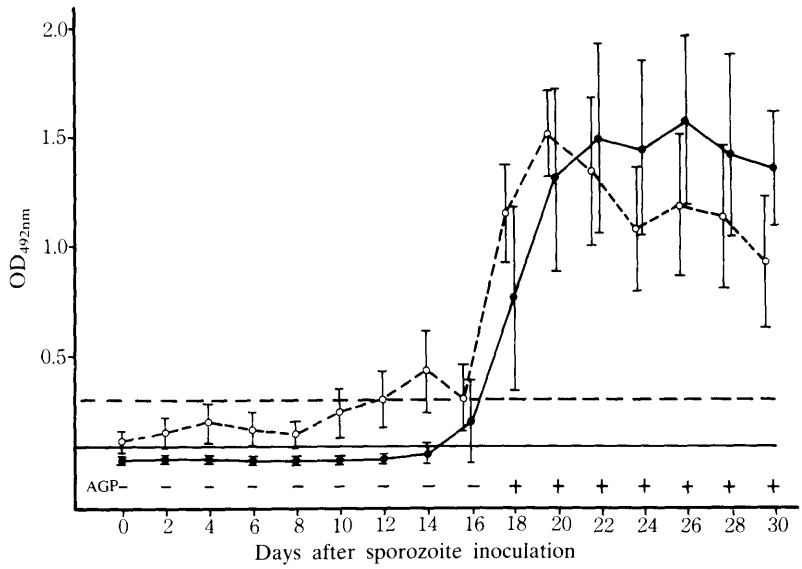

Fig. 2. Antibody response to *L. caulleryi* measured by ELISA in sera from experimentally infected chickens. Eight 93-day-old chickens were inoculated with sporozoite of *L. caulleryi*. Serum samples were collected before and every two days to 30 days after inoculation. ○, IgM antibody; ●, IgG antibody; ⊖; ⊕, Mean and standard deviation; AGP, Agar gel precipitation test; +, Precipitin line was detected; −, not detected; ----------, cutoff point of IgM antibody; ——, cutoff point of IgG antibody.

leryi for 13 months, from June, 1982 to June, 1983 in our laboratory. The serum samples were collected from them once a month at fixed times.

Blood smears, ELISA and AGP test: Blood smears were prepared from one drop of peripheral blood, fixed in methanol for 10 minutes at room temperature, stained with Giemsa stain and observed light microscopically. ELISA and AGP test were carried out in the same manner described previously [5, 7].

Specificity test for the conjugates: Ten chickens wre inoculated with 2 to 6 × 10^3 sporozoits of *L. caulleryi* intravenously and bled from 20 to 23 days after inoculation. Sera were pooled and used for fractionation. The sera were fractionated through a Sephadex G-200 (Pharmacia Fine Chemicals) gel column (2.6 × 32 cm) using 0.01 M Tris-HCl buffer containing 0.15 M NaCl (pH 7.8). Fraction volume was 3.0 m*l* and the absorbance at 280 nm was measured. The demonstration of IgM (μ), IgG (Fc) and IgG (H & L) antibodies against schizonts of *L. caulleryi* in each fraction was done in ELISA.

RESULTS

Specificity of conjugates: Figure 1 (a, b and c) shows the results. The IgG (Fc) conjugate detected 7S globulin and the IgM (μ) conjugate 19S globulin specifically. But the IgG (H & L) conjugate detected both 7S and 19S globulins.

Analysis of sera from experimentally infected chickens: ELISA IgM antibodies against schizonts were detected in some sera on day 10 after sporozoite inoculation (Fig. 2) and reached highest optical density (OD) 3 weeks after inoculation (Fig. 3). After that, they decreased suddenly and maintained a low level until 10 weeks after inoculation (Fig. 3). Contrary to this, ELISA IgG antibodies against schizonts of *L.*

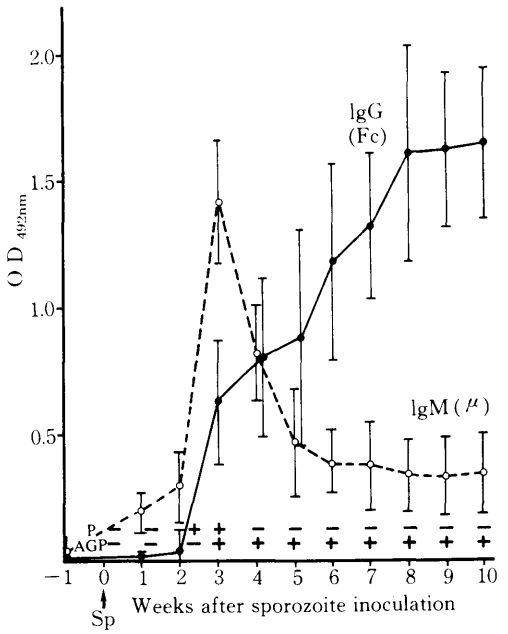

Fig. 3. Antibody response to *L. caulleryi* measured by ELISA in sera from experimentally infected chickens. Ten 28-day-old chickens were inoculated with sporozoite of *L. caulleryi*. Serum samples were collected 1 week before and every week to 10 weeks after inoculation. P, Detection of protozoa in blood smears; AGP, Agar gel precipitation test; +, protozoa or precipitin line was observed; −, not observed; Sp, Sporozoite inoculation.

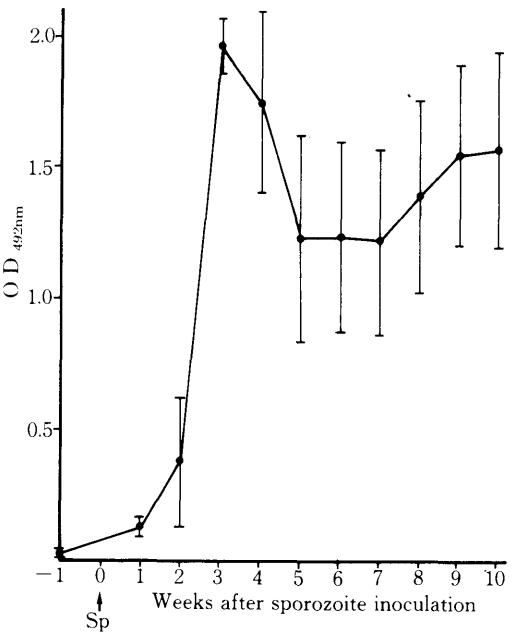

Fig. 4. Antibody response to *L. caulleryi* measured by ELISA using anti-chicken IgG (H & L) conjugate. Sera were the same as in Fig. 3. ⊕, Mean and standard deviation; Sp, Sporozoite inoculation.

caulleryi were detected on day 14 in some infected chicken sera (Fig. 2); later, their OD value gradually rose. They reached a high level 8 to 10 weeks after sporozoite inoculation (Fig. 3).

On the other hand, AGP antibodies against serum-soluble antigen were continuously detected from 18 days to 10 weeks after sporozoite inoculation (Figs. 2 and 3). The protozoa in blood smears could be detected only 2 and 3 weeks after sporozoite inoculation (Fig. 3).

Analysis with anti-chicken IgG (H & L) conjugate: ELISA antibodies against schizonts of *L. caulleryi* detected by using IgG (H & L) conjugate were recognized from 2 weeks after sporozoite inoculation and reached maximum 3 weeks after inoculation. Then they decreased gradually and kept some level. However, from 8 to 10 weeks after sporozoite inoculation, the level increased (Fig. 4).

Analysis of sera from naturally infected chickens: ELISA IgG and IgM antibodies against schizonts were detected with/after detection of protozoa in blood smears (Fig. 5). Subsequently, ELISA IgG antibodies against schizonts showed various high OD values, whereas ELISA IgM antibodies against schizonts decreased, maintaining levels lower than IgG for about one year (Fig. 5).

Protozoa in blood smears were detected only once in each bird from June to August. No protozoa was found in blood smears in other periods (Fig. 5).

DISCUSSION

The anti-chicken IgM (μ) and IgG (Fc) conjugates were found to be specific enough

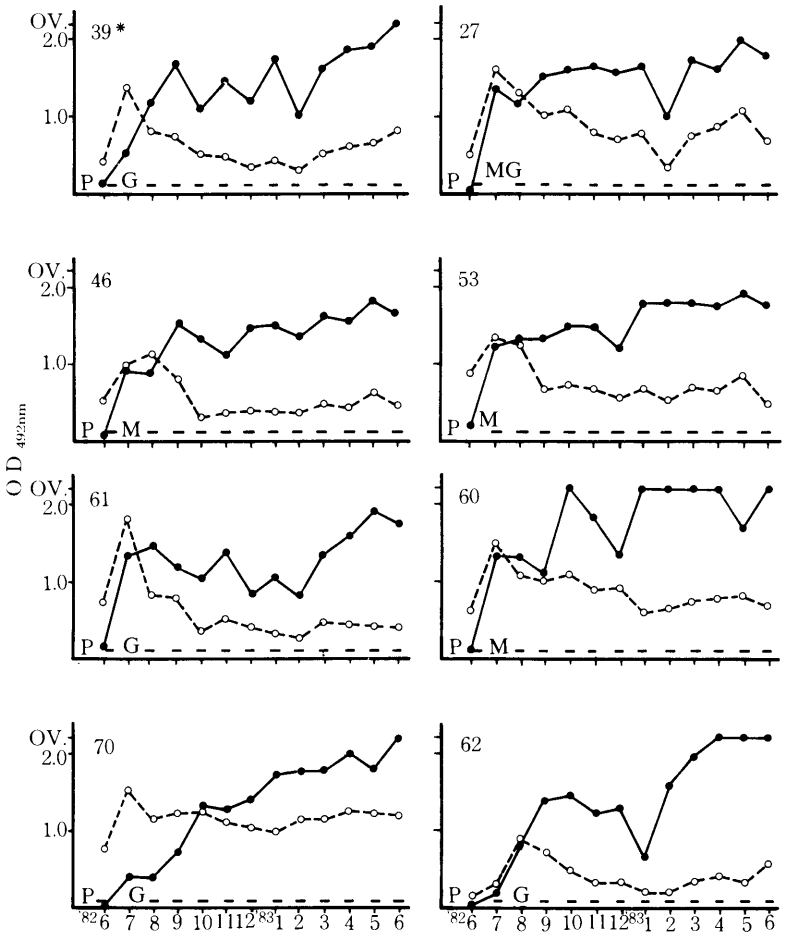

Fig. 5. Antibody response to *L. caulleryi* measured by ELISA in sera from naturally infected chickens. ○, IgM antibody; ●, IgG antibody; P, Detection of protozoa in blood smear; OV, over (>2.0); *, chicken No.; −, not detected; M, Merozoite; G, Gametocyte.

for differentiation of IgM and IgG antibodies against schizonts of *L. caulleryi* when fractionated through a Sephadex G-200 column. On the other hand, anti-chicken IgG (H & L) conjugate was not specific and revealed both IgM and IgG antibodies (Figs. 1 c and 4). This result is supposedly attributed to the fact that the light chain is common to all classes of immunoglobulin [4]. With the anti-chicken IgG (H & L) conjugate, one could detect a wide range of antibody responses to *L. caulleryi* in field cases.

In the AGP test, the antibody against serum-soluble antigen of *L. caulleryi* was detected from 17 days after sporozoite incoculation [12], and the precipitin antibodies in the sera belonged to the 7S class of immunoglobulin [13]. In the present study, the IgG antibody to schizonts of *L. caulleryi* was detected from 14 days after sporozoite inoculation in some infected chicken sera by ELISA. For the first time, the IgM class antibody response to *L. caulleryi* was observed. IgM antibodies against schizonts of *L. caulleryi* were detected from 10 days

after sporozoite inoculation. Based upon these results, the antibody response to *L. caulleryi* was noted earlier than in the AGP test, and the disease would be diagnosed earlier.

In the present experiment, the IgM antibody response to *L. caulleryi* appeared initially in the course of infection, and the IgG antibody predominated over IgM antibodies in the late phase of the experimental period. This type of phenomenon, which has been reported in other diseases [9, 14, 16], is supposed to depend on the B cell development and differentiation [15].

In chickens naturally infected with *L. caulleryi*, both IgM and IgG antibodies against schizonts persisted for nearly one year at various levels. Chickens recovered from infection with *L. caulleryi* show a strong resistance to reinfection [11]. From these results, we could detect the antibody response to *L. caulleryi* every time by ELISA and differentiate the infected from the non-infected chickens. Thus it would be relatively easy to plan a preventive method for leucocytozoonosis in the flock against next epizootics.

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要　　約

Leucocytozoon caulleryi に対する鶏の免疫グロブリンMとGの抗体応答：磯部　尚・鈴木　恭（農林水産省家畜衛生試験場鶏病支場）——ペルオキシンダーゼ標識抗鶏IgM(μ)抗体及び抗鶏IgG(Fc)抗体を作製し，*Leucocytozoon caulleryi* 感染鶏におけるクラス別抗体応答についてELISAを用いて調べた。実験感染鶏血清においてIgM抗体はスポロゾイト(Sp)接種後10日から検出され始め，Sp接種後3週で最も高い値を示した後，漸次低下し，以後低いレベルで推移していた。一方，IgG抗体はSp接種後14日から検出され始め，徐々に上昇し，Sp接種後8～10週で高い値を示した。自然感染鶏血清においては，IgM及びIgG抗体とも原虫検出後，高い値を示すが，その後IgG抗体が高いレベルで翌年度まで推移するのに対し，IgM抗体は低いレベルで推移していた。