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Chronologic change of serum IgG antibody response in chickens reinfected with *Cryptosporidium baileyi*

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Abstract: Eight 2-day-old SPF chickens were each inoculated orally with a single dose of 5×10^5 oocysts of *Cryptosporidium baileyi*, and immunoglobulin G (IgG) antibody responses were chronologically measured by indirect immunofluorescent antibody (IFA) assay. Anti-*C. baileyi* IgG antibody levels remained high (1:106.67 to 1:512.00) for at least 4 months with 330 days of a detectable period. Ten days after the negative conversion, each chicken was re-challenged with 1×10^7 oocysts of the same species. Subsequent infection in 340-day-old individuals caused sudden elevated IgG antibody levels and the titer peaked on day 28 postchallenge inoculation (PCI), at 1:1,024 with a 65 days of detection period. Chickens in primary infection showed oocyst shedding profiles, but did not exhibit any oocyst shedding before or after experimental reinfection.
Key words: *Cryptosporidium baileyi*, chicken, IgG antibody, challenge infection

INTRODUCTION

Naturally acquired *Cryptosporidium* spp. infections have been reported in at least nine different avian hosts, including chickens, turkeys, ducks, quail and etc. Intestinal and respiratory cryptosporidiosis are common among commercially reared broiler chickens. The species believed to be responsible for both respiratory and intestinal cryptosporidiosis of broiler chickens was *C. baileyi* by Current *et al.* (1986). In addition, oocysts of *C. baileyi* were found in the stool of an immunodeficient patient and the coccidia were also detected in various organs of autopsy material (Ditrich *et al.*, 1991).

By day 4 post oral inoculation with *C. baileyi* oocysts in 2-day-old chickens, most parasites occurred in enterocytes of the cloaca and bursa of Fabricius (Current *et al.*, 1986; Rhee *et al.*, 1995). Because of the clear delineation between thymic and bursal lymphoid tissue in chickens, suppression of normal bursal development will result in severe defects in the ontogeny of humoral immune response.

IgG antibody response in chickens infected with *C. baileyi* was examined for only 31 days postinoculation (PI) by Current and Snyder (1988). In 14-day-old chickens inoculated with 1×10^6 oocysts of *C. baileyi*, anti-*C. baileyi* IgG antibodies were detected from day 9 PI, and their titer increased significantly up to day 22 PI (Naciri *et al.*, 1994). No experiments have been performed to clarify a humoral antibody response to *C. baileyi* for a long period, especially a increase and decrease of IgG antibody levels against the protozoa.

The present study was attempted to confirm the chronologic change of IgG antibody response through long-term to understand defense mechanism against challenge infection.

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MATERIALS AND METHODS

C. baileyi oocyst used for the present study was the medium type previously isolated from the domestic chicken, *Gallus gallus*, and serially passed in 2-day-old SPF chickens after an interval of 2 months (Rhee *et al.*, 1991). Eight 2-day-old SPF chickens (Dekalb-Warren, Sex-Sal-Link, male) were each inoculated orally with a single dose of 5×10^5 oocysts. Following inoculation, chickens were maintained in wire bottom cages and given nonmedicated commercial feed and water *ad libitum*. The birds were monitored for *C. baileyi* infection by examination of fecal samples obtained every day, continuing until all infected animals stopped shedding oocysts. Ten days after IgG antibody levels of all birds disappeared, each bird was re-challenged with 1×10^7 oocysts. An IFA test for sera was executed after an interval of about one month excepting for initial and postchallenge stages.

Immunoglobulin G titers were measured according to the method of Rhee (1988). Briefly, 1×10^8 oocysts of *C. baileyi* purified by ether extract method and discontinuous gradient method (Riggs and Perryman, 1987; Arrowood and Sterling, 1987) were suspended in 1.75% BAS, coated evenly onto whole microscope slides, and dried. And then the slide preparations were fixed in cold acetone for 10 minutes and preserved at -70°C until ready for use as an antigen. The antigen slides were reacted with two-fold dilution of serum samples and then with FITC conjugated anti-chicken IgG antibody (Sigma Chemical Co.) in 32-fold dilution. They were examined under fluorescent microscopy.

RESULTS

Results of IFA assay for serum IgG antibodies to *C. baileyi* from 8 individuals are shown in Fig. 1. On days 3 to 38 PI, all chickens exhibited negligible antibody reactions, and seroconversion was not apparent because of a lag phase. The IFA titers were elevated as time progressed. On day 45 PI, all chickens had a mean titer of 1:21.33 and those at days 60, 75, 90 PI being

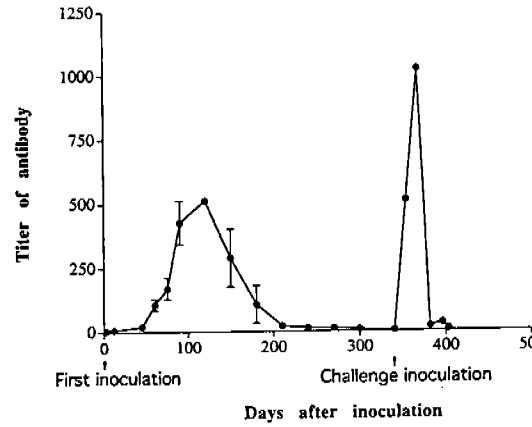

Fig. 1. Serum IgG antibody levels in the primary and secondary antibody responses to *Cryptosporidium baileyi* in chickens reinfected with oocysts.

1:106.67, 1:170.67 and 1:426.67, respectively. The highest titer (1:512) occurred on day 120 PI and declined gradually thereafter. Finally, no specific antibodies to the protozoa were detected in sera from all birds on day 330 PI.

After subsequent challenge infection, there is a rapid response with a very short lag phase, exhibiting the highest titer of 1:1,024 on day 28 PCI. The titers declined rapidly thereafter, eventually became negative for IgG antibody on 65 day PCI.

Meanwhile, the birds experimentally infected with the protozoa started to shed oocysts at 3 day PI and oocyst counts increased gradually thereafter. Oocyst shedding peaked on day 10, subsequently decreased, and fell below detectable levels by day 30 PI. Re-challenged birds did not exhibit any oocyst shedding when observed by Kinyoun's modified acid-fast staining method.

DISCUSSION

In this study, anti-*C. baileyi* IgG antibody levels remained high for at least 4 months in chickens primarily inoculated with a single dose of 5×10^5 oocysts of *C. baileyi*, and the antibody levels were detected through about a year. Detectable period (330 days) for IgG antibody from sera in primary infected animals is longer than that (65 days) in reinfected animals. Ungar *et al.* (1989) demonstrated that

IgG antibodies to *Cryptosporidium* in man may be present for at least 1 or 2 years after infection. Campbell and Current (1983) also reported that an IgG antibody response was detectable for at least a year in sera from five subjects with an acquired immunodeficiency syndrome.

Anti-*Cryptosporidium* IgG antibody levels in postchallenge infection are variable depending on species of *Cryptosporidium*. In a *C. parvum* contaminated environment for the first day of life and subsequently penned individually in a steam-cleaned room, lambs reinfected at 30 or 120 days old did not show any significant increase in the anti-*C. parvum* IgG antibody levels when compared with prechallenge and postchallenge opitical densities (Ortega-Mora *et al.*, 1993). Additionally, in BALB/c mice reinoculated with *C. muris* (strain RN 66) on day 55 PI, anti-*C. muris* IgG antibody titers were not elevated on days 3 and 10 PCI (Ohsawa, 1993). Whereas, in the present study, high antibody level (1:1,024) was detected on day 28 PCI, compared to the prechallenge levels.

As *C. baileyi* is confined to an intracellular position within the microvillous border of the mucosal epithelium of the chicken, it is difficult to insist that serum antibodies play a major role in resistance to subsequent challenge. Current and Snyder (1988) revealed that clearance of heavy infection of *C. baileyi* in broiler chickens was accompanied by the appearance of serum antibodies that were detectable by IFA assay using *C. parvum* oocysts as the antigen. In the present study, clearance of oocyst shedding in primary infection did not coincide with high serum antibodies. In this respect, Ungar *et al.* (1991) established the importance of both $CD4^+$ T-cells and IFN-γ production by a $CD4^-$ $CD8^-$ cell in limiting *C. parvum* infection in adult BALB/c mice. Recently, Hatkin *et al.* (1993) reported that cyclosporin A reduce CMI, but not antibody production and that chickens treated with this chemicals were more susceptible to *C. baileyi* infection, which suggested that CMI is more important in resistance to *C. baileyi* than circulating antibody. In light of these findings, it is supposed that marked seroconversion of IgG antibody for a long duration as well as CMI in primary infected chickens is likely to be closely related with the development of acquired immunity to the protozoa.

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=초록=

닭와포자충 재감염닭의 혈청IgG 항체가 추이

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닭에 있어서 닭와포자충의 재감염에 대한 방어기전을 이해하기 위하여 장기간에 걸쳐 혈청IgG항체가를 경시적으로 조사하였다. 2일령의 SPF 병아리(Dekalb-Warren, Sex-Sal-Link)에 5×10^5개 그리고 IgG가가 정상으로 복귀한 다음 10일인 340일령에 1×10^7개의 닭와포자충의 오오시스트를 경구투여하고 나서 채혈하여 간접형광항체법을 이용하여 혈청IgG 항체가를 측정하였다. IgG가는 감염후 3일부터 38일까지는 대조군과 차이가 없었으나 45일부터 1:21.33으로 상승하기 시작하여 120일에 1:512로 최고치에 이른다음 점점 떨어져 330일에 1:4로 정상치에 이르렀다. 도전감염후 7, 14, 28일에 각각 1:16, 1:512, 1:1,024로서 점점 높아져 정점에 이른다음 급격히 떨어져 63일에 정상으로 복귀하였다. 한편, 대조군의 항체가는 모두 1:2~1:4의 범위에 있었으며 이 원충의 도전감염전 및 후에 있어서 분변으로부터 집충시켜 제작한 Kinyoun 항산염색표본에서도 오오시스트를 전혀 검출할 수 없었다. 분변내의 오오시스트의 소실 시기와 최초 감염에 있어서 IgG가의 피이크는 일치하지 않았다.

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