Protective Effect against Intraerythrocytic Merozoites of Theileria sergenti Infection in Calves by Passive Transfer of Monoclonal Antibody

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Japanese bovine theileriosis is caused by Theileria sergenti (T. sergenti), and brings about mild hyperthermia and anemia in cattle. Previously we produced 13 monoclonal antibodies against the intraerythrocytic merozoites (piroplasms) of T. sergenti. Western blot analysis showed that 6 hybridomas tested recognized polypeptide with molecular weight of 32,000 or 23,000 (32 k, 23 k) [2]. Monoclonal antibodies recognized 32 k polypeptide was found to bind to surface of merozoite by immunoelectron microscopic study [7]. Thus, we attempted the protective effect against piroplasms of T. sergenti infection in calves by passive transfer of the monoclonal antibody that recognizes 32 k polypeptide.

Asctic monoclonal antibody (Ts-MAb) was generated by intraperitoneal inoculation of hybridoma (23C11) [2] into BALB/c nu/nu mice. Four healthy splenectomized Holstein calves, about 4 months old were used. The Chitose stock [8] of T. sergenti, the released merozoites used for infection, was prepared from intraerythocytic merozoites of T. sergenti [4]. The released merozoites were incubated with either asctic fluid of 23C11 (calves Nos. 1 & 2) or asctic fluid of anti-BLV p24 (calves Nos. 3 & 4) at 37°C for 30 min. Four calves (Nos. 1, 2, 3 & 4) were inoculated intravenously with 3 x 10⁶ per calf. They were also administered with Predonisolone for 7 days beginning on the 1st and 28th day, respectively, after inoculation with T. sergenti. Two calves (Nos. 1 & 3) were injected with asctic fluids of Ts-MAb (30 ml per calf) by intravenous injection, before and after 4 days inoculation of T. sergenti.

The clinical and hematological findings, rectal temperature and clinical symptoms were observed after the inoculation. Erythrocyte count (RBC), leukocyte count (WBC) and packed cell volume (PCV) were conducted according to conventional methods. More than 1,000 erythrocytes were examined to express the rate of parasitemia under Giemsa staining. The number of parasitized erythrocytes is expressed as a percentage of total erythrocyte counted. Antibody against T. sergenti was detected by indirect fluorescent antibody assay (IFA) and by enzyme-linked immunosorbent assay (ELISA) [5]. Luminol-dependent chemiluminescence (CL) assay [3] was used to measure activation of monocytes in the peripheral blood when challenge exposed with zymosan and merozoite as phagocytic stimulants.

Figure 1 shows the changes in hematological examination after inoculation with T. sergenti. In calves Nos. 1 and 2 no parasitemia was observed and no clinical signs were detectable for 60 days after inoculation. Parasitemia occurred in calf No. 3, but the maximum levels were constantly below 0.01% on the 43th to 50th day after inoculation. On the other hand, in calf No. 4, clinical signs such as hyperthermia, anemia and icterus were detectable, although a slight decrease in erythrocyte count was seen. Leukopenia was also observed. The parasitemia reached a maximum level of 7.2% on the 50th day.

Antibody titers were tested by IFA and ELISA. In calves Nos. 1, 2 and 3, no changes were observed in IFA titer and ELISA value. In calf No. 4, changes in antibody titers by IFA and ELISA were observed in accord with parasitemia. Antibody appeared in serum about 40 days after inoculation and increased thereafter. The peak of the IFA titer (1:3200) and ELISA value (0.53) were recorded on the 55 day after inoculation. IFA titers were also similar to those shown

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Macrophage activity was tested by CL assay at 40 and 67 days after challenge. A measurable oxidative burst was observed in all 4 calves at 40 days when macrophages were challenge-exposed with opsonized merozoite. Opsonization of merozoite with its own serum obtained at 67 days induced strong CL response in macrophages from calf No. 4 compared to those from calves Nos. 1 to 3.

As a result, it was suggested that the protective effect against piroplasms of *T. sergenti* infection in calves might be introduced by passive transfer of monoclonal antibody. However, the exact mechanism by which the monoclonal antibody bestows this protective effect remains to be elucidated.

In previous communications, it was reported that humoral antibody and cell-mediated immunity participated alone or in combination in the defense mechanisms of against *Babesia ovata* and *T. sergenti* infections [1, 6, 9].

Previously we tested the luminol-dependent CL assay to assess the role of macrophages in *T.*
sergenti-infected calves and found the activation of macrophages within one month after infection. Moreover, opsonized merozoites with immune sera induced strong CL response compared to that of non-opsonized merozoites (submitted). Thus it seems that combination of specific antibodies against merozoites and activated macrophages is the most important way to prevent inversion of erythrocytes by merozoites.

REFERENCES


要約

抗 Theileria sergenti モノクローナル抗体による小型ピロプラズマ病の防御効果 (短報): 田中雅之・階谷年昭・岡部達二・川本哲1)・高橋清志1)・小沼操2)・川上善三2) 佐々木文雄 (微生物化学研究所、経農学園大学1) 家畜内科学教室、2) 家畜微生物学教室) における小型ピロプラズマ病原体である Theileria sergenti (Ts) のメロゾイトに対するモノクローナル抗体による牛における小型ピロプラズマ感染に対する防御効果について検討した。その結果, Ts メロゾイドの32キロダルトンの分子量を認識するモノクローナル抗体 (23C11) の牛への移入は、小型ピロプラズマの発病抑制効果が認められた。