Opsonization of Merozoites with Sera from *Theileria sergenti*-Infected Calves

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*Theileria sergenti* (*T. sergenti*) infection is one of the most economically important tick-borne diseases of grazing cattle in Japan. The role of cellular and humoral immunities in the defence of cattle during infections by *Theileria parva* has been widely investigated [3, 8].

In *T. sergenti*-infections, such investigations are limited. However, the effectiveness of anti-merozoite antibodies was clearly demonstrated by passive transfer of monoclonal antibody against merozoites of *T. sergenti* [13]. Several reports concerning cellular immune responses in *T. sergenti*-infected calves have shown the importance of macrophages in controlling the progression of parasitaemia and clinical symptoms [10, 12]. Luminol-dependent chemiluminescence (CL) responses are widely used to study oxidative events of macrophages. Previously we tested macrophage activity in *T. sergenti*-infected calves by CL responses and found the significant macrophage activation within one month after inoculation [1]. During the course of the CL response, we observed increase of the response when stimulant was opsonized with immune sera. In the present study, we tested the effect of opsonization of merozoite with serum components in order to understand the role of macrophage in the infected calves.

Luminol-dependent CL responses of macrophage oxidative events were measured by the method previously described [1, 14]. Briefly, macrophages in peripheral blood mononuclear cells (PBMC) were suspended in modified Hanks balanced salt solution (HBSS) without phenol red, and adjusted to $1 \times 10^6$ macrophages/200 $\mu l$. Twenty $\mu g$ of luminol (Sigma) were added to macrophage preparation of 200 $\mu l$, and CL response was monitored in Biolumat LB9500. Opsonized (op $^+$) or non-opsonized (op $^-$) merozoites were used as stimulant. Cell-free merozoites were prepared from parasitized bovine erythrocytes (Chitose stock) [9]. For opsonization, one volume of purified merozoites ($1 \times 10^9/ml$) was mixed with 3 volumes of *T. sergenti*-positive bovine serum (p-1) (antibody titer 1:10000 by immunofluorescence test) and incubated at 37°C for 1 hr. After incubation, merozoites were washed by centrifugation and adjusted to the original concentration. Twenty $\mu l$ of op $^+$ or op $^-$ merozoite ($1 \times 10^9/ml$) were used as stimulant. Merozoites were also opsonized with following serum materials; *T. sergenti*-negative serum, purified IgG fraction of p-1 and its papain digested IgG (both Fc and Fab portions), fresh calf serum (negative for *T. sergenti*) as bovine complement, and monoclonal antibody against merozoite (23Cl1) [6, 11].

Purified merozoites were opsonized with *T. sergenti*-infected bovine serum and used as stimulant for CL response to compare the responses before and after opsonization. Macrophages used were prepared from individual 8 *T. sergenti*-infected calves. CL responses of all 8 macrophage preparations increased when merozoites were opsonized with immune serum (Fig. 1). Next, we tested the effect of opsonization with various bovine serum components containing IgG fraction, its Fc or Fab portion and bovine complement using 3 individual macrophage preparations. As shown in Fig. 2, the highest response observed was merozoites opsonized with either immune serum or its IgG fraction. Fab portion of the IgG, however, could not enhance the response, indicating the importance of Fc portion of IgG. Bovine complement did slightly increase the response in one calf (Fig 2 c’)

Thus we tested the effect of complement in opsonization. Merozoites were mixed with inactivated p-1 serum and bovine complement (final concentration of 5% or 10%), stood for 1 hr at
37°C, and after washing used as stimulant. Addition of bovine complement to stimulant showed an increase of the response (5%, 1280±152 cpm, n=7; 10%, 1560±230 cpm, n=7) compared to that without complement (724±36 cpm, n=8).

Opsonization of merozoites, with IgG fraction of mouse monoclonal antibody against merozoite, 23C11 did not increase the response (mean cpm of merozoites alone and merozoites plus 23C11 were 277 and 319, respectively, n=3). However, merozoites opsonized with 23C11 plus sheep anti-mouse IgG antibody showed an enhancement of CL response (mean cpm, 1773, n=3), similar to that opsonized with bovine immune serum (2170 cpm, n=1).

CL is a reliable method for assessing phagocytic function. Studies have shown that CL parallels oxygen consumption, in vitro phagocytosis and killing of bacteria [4]. In Plasmodium falciparum (P. falciparum) infections, there are many reports concerning a cooperative effect of anti-malaria antibody with human monocytes. Phagocytic activity of human monocytes for P. falciparum infected erythrocytes was greatly enhanced by the presence of immune sera [2, 5], and the immune sera induced marked specific inhibition of parasite growth in the presence of human blood monocytes [7]. The phagocytosis enhancing activity of the immune sera was associated with IgG fraction [5]. In bovine system, passive transfer of specific antibody to merozoites showed a protective effect against piroplasms of T. sergenti-infection [13]. Although the exact mechanism of this protection is not known, present results and the evidence obtained in malaria infection suggest that the combination of IgG and bovine macrophages may prevent the invasion of merozoites to erythrocytes. To understand protective mechanisms, phagocytic activity against free merozoites and parasitized erythrocytes by bovine monocytes in the presence of immune sera is now under progress.

REFERENCES

要約

Theileria sergenti 感染牛血清によるオプソニン効果（短報）：新川 千一・小沼 操 1)・松葉隆司・桐沼利雄・高橋清志・川上善志（酪農学園大学獣医学部，1) 北海道大学獣医学部）——Theileria sergenti 感染牛マクロファージの機能測定をケミカルイネッセンス (CL) 法により行った。測定の際、赤内型メロソイドをオプソニン化し stimulant として CL 反応を調べた。T. sergenti 隠性牛血清ないしはその IgG でオプソニン化すると活性の増強がみられた。しかし、IgG を Fab, あるいはFcフラグメントにするとオプソニン効果は消失した。またウシ補体でも弱いオプソニン効果を認めた。