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FULL PAPER

Passive immunization with monoclonal antibodies : effects on *Haemaphysalis longicornis* tick infestation of BALB/c mice

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Abstract

Tick vaccine development plays an important role in current tick control strategies. Previously, we have produced three different isotypes of monoclonal antibodies (mAbs) which recognized a midgut protein of adult *Haemaphysalis longicornis*. These mAbs, typed as IgG1, 2a, and 2b, reacted with a 76 kDa surface protein of midgut cells. We speculated that the 76 kDa protein may be an unknown antigen for a tick vaccine and the three mAbs may work as probes to clone the protein. In this study, to test whether these three isotypes have anti-tick effects and if so which works more effectively, we conducted passive immunization in BALB/c mice with each of the mAbs, and infested the mice with adult ticks. All isotypes significantly reduced the number of hatched larvae, compared to controls, however, no differences in the magnitude of the reduction were observed among the three.

Key words : *Haemaphysalis longicornis* ; midgut ; monoclonal antibodies ; passive immunization

Introduction

An ixodid tick, *Haemaphysalis longicornis* is a bovine ectoparasite that causes economic losses in Asia, mainly as a vector of Theileriosis and Babesiosis, due to the high costs required to control the parasite[6, 13)]. Acaricides are the principal tick control method but they have the effect of contaminating foods and the environment, and ticks tend to develop resistance to them[12, 14)]. Tick vaccines inducing host protective immunity

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have been developed to resolve these problems. Ticks have two kinds of vaccine candidate antigen ; exposed antigen which is secreted from salivary gland and exposed to the host immune-system, and concealed antigen which is present in internal organs such as midgut, and thus, does not naturally induce a host immune-response[9]. We have previously cloned and confirmed *H. longicornis* salivary gland antigens, p29 and HL 34, as vaccine candidates[7,15]. They showed efficient protection against larval and nymphal ticks, and partial protection against adults. On the other hand, it was found that a *Boophilus microplus* midgut antigen has protective effects against adult ticks, but not against larvae[3,4]. These different sensitivities of host-acquired resistance against tick antigens suggest that a cocktail vaccine containing more than one antigen would elicit a co-operative protective effect[17].

To identify concealed antigens of *H. longicornis*, we have produced 8 hybridoma clones secreting mAbs of three different isotypes, as specific probes against midgut protein[8]. These mAbs are IgG1, 2a, 2b isotypes, respectively, and all reacted with the same 76 kDa protein distributed in the plasma membrane and cytoplasmic region of midgut digestive cells[8]. This previous study reported that adult ticks,when they infested mice producing ascites of the mAbs reactive with the 76 kDa antigen, caused the destruction of midgut cells and high mortality[8]. This tick challenge test has been conducted with only the IgG1 isotype mAb, not yet with IgG2 isotypes. On vaccination with recombinant Bm86 which is a midgut antigen of ticks, it was reported that different isotypes of IgG recognized the same Bm86 antigen, but had an altered efficacy of protection against *B. microplus*[10]. Thus, in the present paper, to confirm the anti-tick effects of mAbs, we conducted passive immunization using different IgG isotypes of mAbs against a *H. longicornis* midgut antigen, in a BALB/c mouse model.

Materials and Methods

Ticks and preparation of mAbs

Haemaphysalis longicornis (Okayama strain, female and parthenogenesis) obtained from the National Institute of Animal Health (Japan) were propagated by infestating naive Japanese white rabbits kept free of pathogens. These ticks were used for challenge tests and histological assays. In previous studies, mAbs raised against midgut digestive cells of adult ticks were typed as IgG1, IgG2a and IgG2b[8]. Aliquots from pooled ascites were purified by chromatography on a protein G Sepharose column (Amersham Pharmacia Biotech, UK) and the concentration of each IgG isotype was measured.

Passive immunization and challenge

Eighteen to nineteen-week-old female BALB/c mice (Charles River, Kanagawa, Japan) were intraperitoneally immunized with ascites containing mAbs, daily, for 5 days. Water and food pellets for mice were supplied *ad libitum*. Three groups, each consisting of 4 mice, were inoculated with different IgG isotype ascites, and 4 mice with control ascites containing unrelated mAbs. To test the effect of the mAbs at high dose, mice of each group were inoculated with ascites containing a total of 0.5 mg of IgG, but the 2a group was not tested at this dose, because of the low concentration of IgG. For the low dose test, ascites containing a total of 0.2 mg of IgG were introduced into mice. After passive immunization on day 0, mesh sacs containing adult ticks were fixed on the backs of the mice. Seven or eight adult ticks fed on one mouse. For this passive immunization test, adult but not nymphal ticks were used because a previous

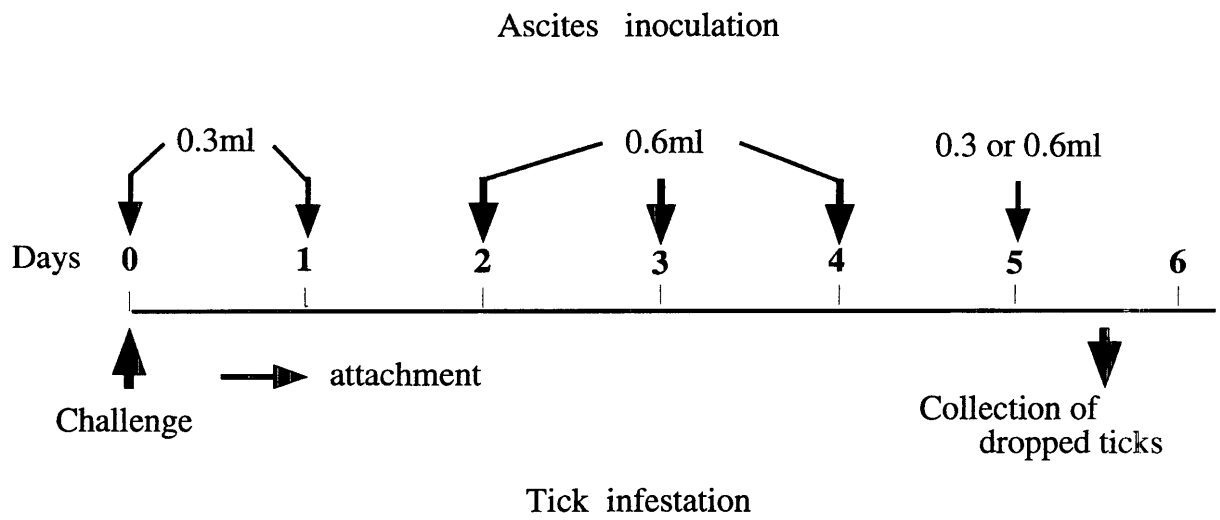

Fig. 1 Schematic representation of passive immunization. Ascites of each isotype mAb was diluted to contain at least 50 μg/0.3 ml of IgG, and mice were immunized with 0.3 or 0.6 ml of ascites every day, as a high dose mAbs test (described in Materials and Methods). In a similar way, a low dose test was conducted. Each IgG concentration was adjusted to 20 μg/0.2 ml, and mice were immunized with 0.2 or 0.4 ml of ascites every day.

study showed that none of the mAbs reacted with nymphal midgut protein[8]. The observation of ticks and immunization of mice were conducted on a daily basis as outlined in Fig. 1.

Effects of passive immunization against ticks

After the last immunization, all ticks were collected and weighed. Anti-tick effects were determined by visual observation of a red discoloration as well as failure to engorge (Fig. 2). It was assumed that ticks suffered from gut damage if they had a red discoloration of the hemolymph due to leakage of hemoglobin or erythrocytes across the gut[3]. To confirm the anti-tick effect histologically, engorged and non-engorged ticks in each group were embedded in Tissue-Tec O.C.T.compound (Sakura Finetechnichal, Japan) and quickly frozen in liquid nitrogen. Frozen sections, 10 μm thick, were prepared using a cryostat (CM 3050, Leica, Nussloch, Germany) and stained with 0.2% toluidine blue for observation un-

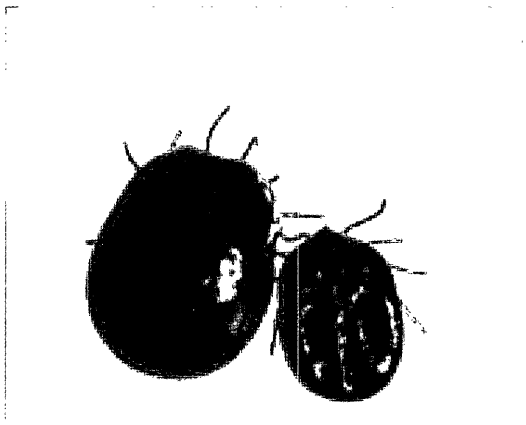

Fig. 2 Adult ticks after passive immunization on day 5. The left tick ingested unrelated IgG1 as a control, while the right tick ingested specific IgG1. The right tick showed red discoloration and a smaller engorged body than the control, as anti-tick effects.

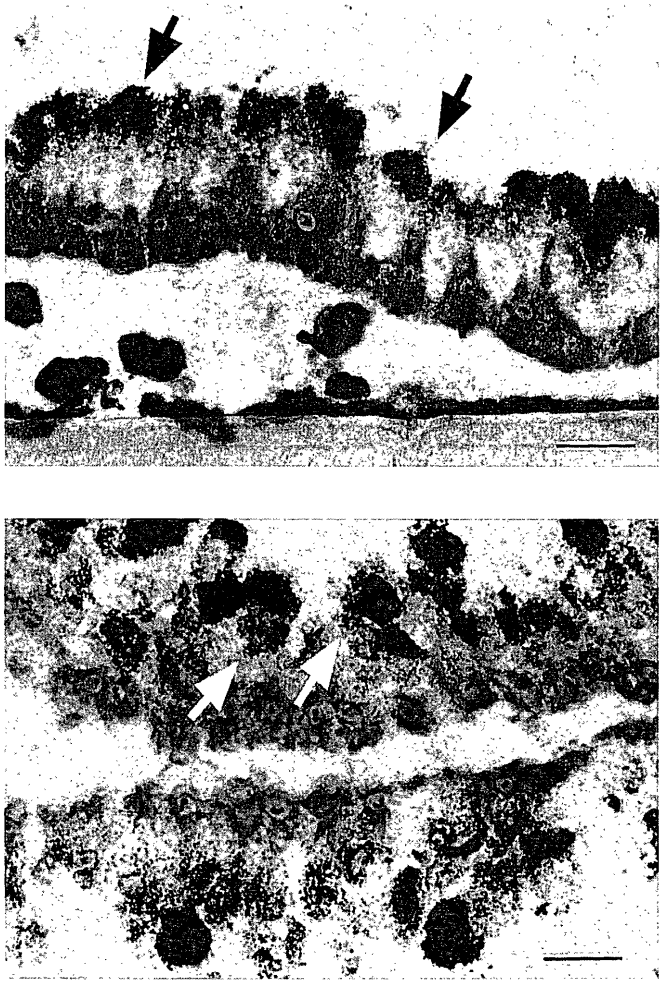

Fig. 3 Toluidine blue-stained sections showing the destruction of midgut cells.
The sections were obtained from adult ticks in Fig. 2. The black arrows in (A) show normal digestive cells in the midgut of a tick that ingested unrelated IgG1. White arrows in (B) show digestive cells which are destroyed due to ingestion of specific IgG1. The scale bars are 50 μm.

der a light microscope[8]. Meanwhile, collected ticks were maintained at 25℃ in an atmosphere with 90% relative humidity for oviposition and larvae hatching. Hatched eggs and larvae were weighed or counted.

Results

Anti-tick effects of the mAbs and differences in the magnitude of effects among the isotypes were examined by performing passive immunization experiments. All exposed ticks were collected by day 6. Fig. 1 shows the schedule. Fig. 2 shows the ticks collected, an adult tick which failed to engorge normally and an engorged tick, right and left, respectively. Histological differences in the effects of the mAbs were observed in the midgut of non-engorged ticks compared with normal engorged ticks (Fig. 3).

The results of passive immunization against adult ticks with the mAbs at high dose are summarized in Table 1. Significant

effects such as a reduction in engorgement, capacity to reproduce, and hatching were observed. Both the IgG1 and 2b isotypes elicited a reduction in engorgement and egg weight, and especially the number of hatched larvae. The weights of engorged adults were reduced 18.1% (IgG1) and 7.3% (IgG2b) compared to the controls using unrelated mAbs (Table 1). Rates of reduction (%) in the weight of eggs caused by these mAbs were 15.0% (IgG1) and 22.0% (IgG2b). Reductions in the number of hatched larvae were remarkable, at 62.0% (IgG1) and 61.1% (IgG2b). These results were for passive immunization at high dose. Subtype IgG2a was not tested at this dose (described as Materials and Methods). On passive immunization at a low dose, no reduction in engorgement or egg weight was observed as compared to controls. However, remarkable reductions in the number of hatched larvae were observed : 70.1% (IgG1), 53.0% (IgG2b) and 55.1% (IgG2a) compared to controls (data not shown).

Discussion

The midgut antigen has been considered a concealed type and has better immunogenicity than antigens exposed during tick infestation[9]. To obtain a vaccine candidate from midgut proteins of *H. longicornis*, we produced monoclonal antibodies (mAbs) of three different isotypes, IgG1, 2a and 2b, all of which reacted with a 76kDa plasma membrane protein in the midgut. Based on our preliminary experiment, it was predicted that the mAbs would have a protective effects, perhaps causing the mortality of adult ticks[8]. In this study, we conducted passive immunization experiments to determine the anti-tick effects of the mAbs in detail. The results showed remarkable reductions in the number of hatched larvae with all mAbs (Table 1).

In previous reports, the IgG1 rather than IgG2 isotype of tick midgut-specific antibodies induced midgut damage, and this reaction required complement, as observed in ticks feeding on immunized cattle[4,14]. However, in experiments using mice, it has been documented that IgG2a is more effective than IgG1 in Fc-

Table 1. Effects of high dose mAbs inoculated into adult ticks

Isotype	Tick specificity	No. of[a] ticks	Mean weight[b] (mg / adult)	Mean egg weight[c] (mg / adult)	No. of hatched[d] larvae (per adult)
IgG1	−[e]	29	108.2	48.0	967.5
	+	33	88.6 (18.1)	40.8 (15.0)	367.2 (62.0)
IgG2b	−[e]	27	110.8	53.1	1132.1
	+	21	102.7 (7.3)	41.4 (22.0)	440.3 (61.1)

( ) The number shows extent of reduction (%) compared to controls.

a) Total number of adult ticks attached and engorged on mice (4 mice were inoculated with specific mAbs and 4 mice with unrelated mAbs).

b) Mean weight of index[a] shows mean weight of engorged adult ticks.

c) Total weight of eggs divided by index[a] shows weight of eggs produced by one engorged adult tick.

d) Total number of larvae hatched from eggs produced by inoculated adults, divided by index[a]. This index means number of larvae hatched from eggs produced by one adult tick.

e) Control groups were inoculated with unrelated mAbs of isotype IgG1 or IgG2b, respectively.

mediated functions, such as Ab-dependent cell -mediated or complement-dependent cytotoxicity, in anti-tumor effects[1,5], and in blocking the transmission of *Plasmodium falciparum* or *P. gallinaceum*[2,11]. In the present study, it can be speculated that the anti-tick effects of the mAbs work independent of complement, because different isotypes of mAbs showed similar anti-tick effects (Table 1). This is consistent with a previous report that antibodies against the Bm86 antigen work independent of complement[16]. It is thought that Bm86 is a cell surface receptor related to endocytosis[14], and antibodies against the molecule inhibit endocytosis, resulting in reduced tick feeding[14]. However, in another study using yeast derivatives and adjuvants, increased levels of anti-Bm86 IgG1 inducing more protection associated with complement have been observed in calves[10]. Such a system could be used to develop tick vaccine, to increase protective immunity with regard to animal species.

The function of the 76 kDa protein on the surface of midgut cells of *H. longicornis* remains unclear, but it might be similar to that of Bm86. Other than a reduction in the number of hatched larvae, passive immunization had little significant anti-tick effect (Table 1). This may be for several reasons, for example, test conditions, concentrations of mAbs, and most likely the immunogenicity of the 76 kDa antigen which is insufficient for protection. However, results of preliminary experiments[8] imply that if increased quantities of IgGs against the 76 kDa antigen are raised, the antigen turns out to be a better vaccine candidate. In future, this antigen may, therefore, be a useful component of an envisaged cocktail vaccine.

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