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Original Article

The biological activity of ABA-1-like protein from *Ascaris lumbricoides*

Riho Muto, Shinjiro Imai, Hiroyuki Tezuka, Yuko Furuhashi and Koichiro Fujita

Institution: Section of Environmental Parasitology, Department of Health Development, Division of Public Health, Graduate School, Tokyo Medical and Dental University

The elevation of non-specific IgE (total IgE) in *Ascaris* infection can be seen one week after infection, and reaches a peak after approximately two weeks. It has been reported that ABA-1 protein is the main constituent in the pseudocoelomic fluid of *Ascaris suum*. To investigate the effect of the ABA-1-like protein from *Ascaris lumbricoides* (ALB), the cDNA was cloned by reverse transcriptase polymerase chain reaction, using original primers based on the consensus sequences of ABA-1 and TBA-1, that is an ABA-1-like protein from *Toxocara canis*. The clone was sequenced, we constructed the recombinant polyprotein of ALB (rALB14 and rALB7) based on the ALB sequence, and rALB was administrated to BALB/c mice. Fourteen days after inoculation with rALB14 which is the full length of ALB, the elevation of total IgE which we supposed to contain non-specific IgE was observed, and the results were as we expected. Furthermore, in an *in-vitro* experiment, we confirmed that the spleen cells proliferated when stimulated by rALB14 and concanavalin A. Therefore, the whole conformation of ALB is considered to be involved in the elevation of non-specific IgE, and is involved in the activation of T cells.

Corresponding Author: Dr. Koichiro Fujita
Institution: Section of Environmental Parasitology, Department of Health Development, Division of Public Health, Graduate School, Tokyo Medical and Dental University 1-5-45 Yushima Bunkyo-ku Tokyo 113-0034, Japan
Tel: 81-3-5803-5193
Fax: 81-3-5684-2849
E-mail: fuji.mzoo@med.tmd.ac.jp
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Introduction

Parasitic helminth infection such as *Ascaris* infection, is characterized by an elevation in levels of parasite non-specific IgE antibodies, which begin to elevate one week after infection, and reach a peak about two weeks *after* the elevation of parasite specific IgE antibodies[1–2]. It is known that parasite specific IgE antibodies act to exclude parasites from the host[3], whereas, non-specific IgE antibodies are thought to be produced for the purpose of evading the immune system of the host[4]. Thus it is important to clarify whether non-specific IgE antibody production in the infection of parasites is involved in the evasion system of those parasites. However the actual system of production of non-specific IgE antibodies in the definitive host in instances of natural infection, remains to be investigated. Previously, Uchikawa *et al*. investigated non-specific IgE production occurring when mice were peritoneally inoculated with an excretory-secretory (ES) product from *Nippostrongylus brasiliensis*[5]. This result suggests the possibility that the non-specific IgE antibody is produced by a molecule within the parasite.

ABA-1 is the body fluid allergen-1 of *Ascaris suum*; it is found mainly in the pseudocoelomic fluid of *Ascaris*[6], and is targeted by an IgE antibody response in infected humans and rodents[7]. ABA-1 homologues have been identified in various nematodes eg. *A. lumbricoides*[8]. First, ABA-1 is synthesized as large polypeptides, and cleaved by protease into approximately 14-kDa polyprotein. In nematodes, these

polyproteins are especially termed nematode polyprotein allergen (NPA)[9]. ABA-1 has a unique construction containing repeating units with tetra-basic amino acids (RRRR) at the carboxy terminal[10]. Each unit shows the homologues of its amino acids: the positions 13 to 68 and 69 to 124 give 25% identity and 60.7% similarity between the sequences of the two putative domains.

Recently, we have found that NPA from *Dirofilaria immitis* (DiAg) induces non-specific IgE antibodies following peritoneal inoculation in BALB/c mice [H. Tezuka, submitted to *Infection and Immunity*]. We speculate that this observation might support the possibility that NPA is involved in the evasion system of the parasite, but it is still unclear whether NPA like ABA-1, is involved in non-specific IgE production. Therefore, to determine whether the ABA-1-like protein from *A. lumbricoides* (ALB), along with DiAg, is involved in non-specific IgE production, we constructed two types of recombinant ALB (rALB14 and rALB7) by *E. coli*. rALB14 covers the full length of ALB and rALB7 covers a half-length of ALB, following the pattern of conformation of ALB. We measured the total IgE containing non-specific IgE following a single peritoneal inoculation of these recombinants to BALB/c mice. In an *in-vitro* experiment, we also measured both cell proliferation and the production of interleukin 4 (IL-4) of naive mice spleen cells when stimulated by rALB14 or rALB7.

Materials and methods

Parasite materials and RT-PCR

An adult *A. lumbricoides* worm was obtained from a 30 year-old Japanese man who was diagnosed with ascaridiasis, and it was frozen at –80°C upon excretion. Total RNA was then isolated from 35 mg of frozen worm by RNeasy (QIAGEN, Chatsworth, CA) according to the manufacturer's instructions.

A reverse transcriptase polymerase chain reaction (RT-PCR) was performed by an RT-PCR kit (RT-PCR high, TOYOBO, CO., LTD., Osaka, Japan) using random primers for the reverse transcription and oligonucleotide primers for PCR. Oligonucleotide primers were based on the consensus sequence of ABA-1 and TBA-1 (Fig.1. (a)). The sequence of ASPS1 is 5´-TTAAGCATGTTGTTGG-3´ and ASPA2 is 5´-AATTTCGCGGCATCCGCC-3´. The PCR cycle condition was 35 cycles at 94°C for 30 sec/ at 60°C for 30 sec /at 72°C for 90 sec, then at 72°C for 9 min.

Cloning and sequencing of DNA

The amplified DNA fragment was cloned into pCR 2.1vector by a TA cloning kit (Invitrogen, San Diego, CA) according to the manufacturer's instructions. Several white colonies were selected for subcultures, and the plasmid DNAs were extracted by the alkaline extraction procedure. These plasmid DNAs were digested with EcoRI to detect the inserts, and one of the inserts was sequenced by the dideoxy chain termination method with standard oligonucleotide primers.

Construction of recombinant polyprotein

The recombinant polyprotein (rALB14) was prepared as follows. Since the amplified DNA was thought to be the linked fragment of the C-terminal half of the former unit and the N-terminal half of the latter, the insert was amplified by PCR using the primers ALP1S/ALP1AS and ALP2S/ALP2AS. Primer ALP1S was 5´-GGCTCTTAAGCATGTTGTTGGGG-3´ (forward) containing AflII site, primer ALP1AS was 5´-AGTGGATCCTTACCTCCTTCGTCG-3´ (reverse) containing BamHI site, primer ALP2S was 5´-CGACATATGCATCATTTCACTCTTGAAAGC-3´ (forward) containing NdeI site, ALP2AS was 5´-AATTCTTAAGAATTTCGCGGCATCCGCCC-3´ (reverse) containing AflII site. The fragment amplified by ALP1S/ALP1AS was digested with BamHI and AflII, and ALP2S/ALP2AS was digested with NdeI and AflII. Those two fragments were then ligated by a ligation kit (TAKARA SHUZO, CO., LTD., Shiga, Japan), and after ligation, the fragments were amplified by PCR using the primers ALP2S /ALP1AS. PCR cycle condition was 25 cycles at 98°C for 15 sec/at 55°C for 2 sec/at 74°C for 30 sec using KOD polymerase (TOYOBO). These PCR products were digested with NdeI and BamHI, and 1.0% agarose gel electrophoresis was carried out to detect the products. A 399-bp fragment was then purified by Geneclean II (BIO.101, Vista, CA).

The recombinant N-terminal half of rALB14 (rALB7) was amplified by PCR with the primer ALP2S (forward) and with the primer ALP3AS (5'-AGTGGATCCATAATTTCGCGGCATCC-3´; reverse) containing BamHI site and was finally purified by Geneclean II.

The ALP2S/ALP1AS and ALP2S/ALP3AS fragments were subcloned into the pET3b vector digested with BamHI and NdeI, and were used to transform *E.coli* DH5α. The plasmid DNAs from the selected colonies were then extracted by the alkaline extraction procedure and they were used to transform *E.coli* HMS174. For analysis, these transformants were cultured with M9ZB medium (SIGMA CHEMICAL Co., St.

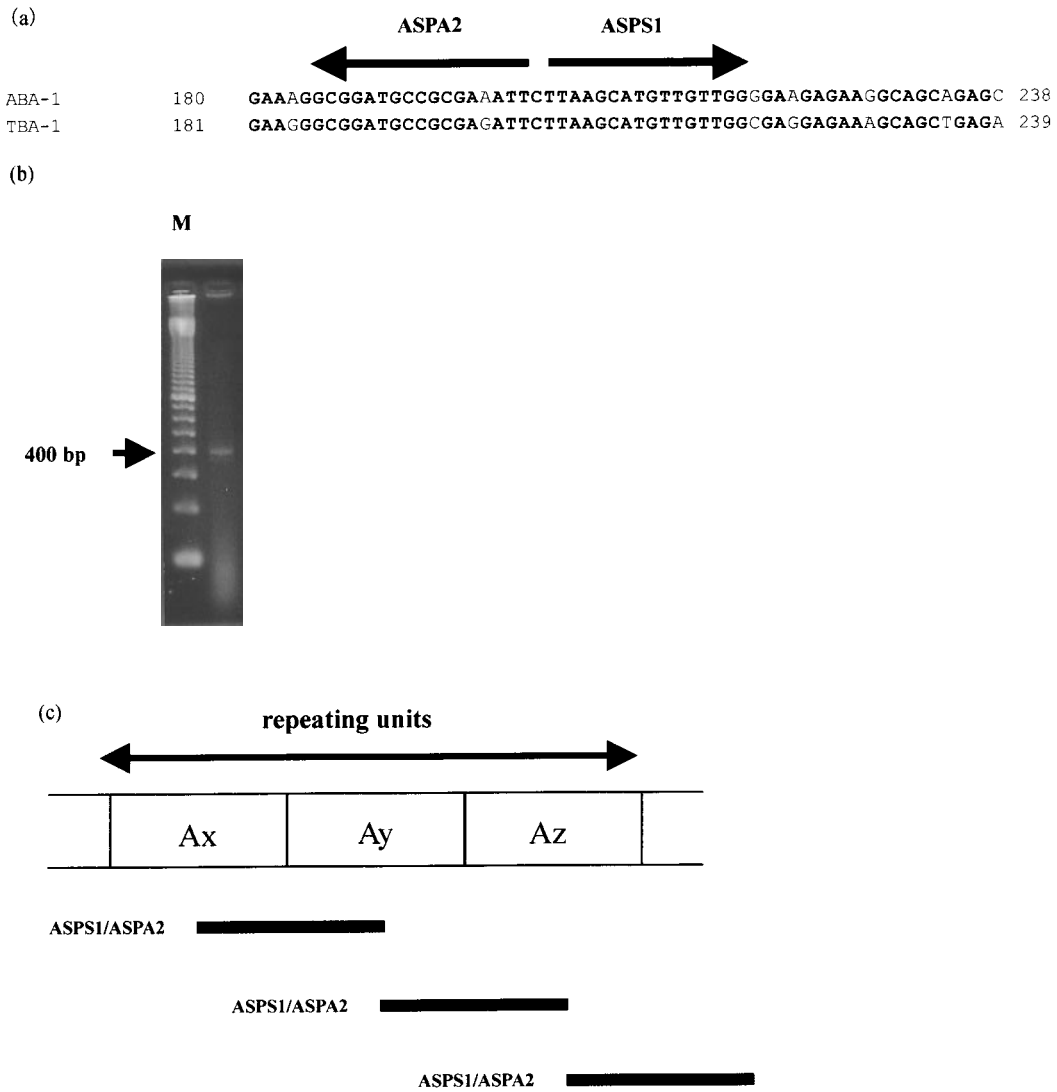

Fig. 1. (a) The comparison of polyprotein DNA sequences from *A.suum* (ABA-1) [10] and *T.canis* (TBA-1) [15], and the design of primers. ASPS1 and ASPA2 indicate PCR primers. Identical nucleotide residues are shown in bold. (b) PCR product amplified by RT-PCR with ASPS1/ASPA2. M indicates a size marker. (c) The scheme of cDNA repeating units of ABA-1 polyprotein. Ax, Ay and Az respectively indicate the monomer units of ABA-1. ASPS1/ASPA2 indicates the fragments amplified by PCR with primers ASPS1/ASPA2 .

Louis, MO) and cells were suspended in 200 μl of 8 M urea, 0.1 M Tris-HCl pH 8.8 and then sonicated. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by Phastsystem using Homogeneous 20 (Amersham Pharmacia Biotech, Buckingham, UK) according to the manufacturer's instructions.

Recombinant proteins were purified in the following procedure. Cultured cells were washed with 50 mM HCl, and the recombinant proteins were extracted by 100 mM HCl. The neutralized extracts were applied on Superdex 200 (Pharmacia) with PBS[11].

SDS-PAGE was performed to detect the purified polyprotein and finally, contaminants of pyrogen from HMS174 were removed from the concentrated rALB solution by immobilized Polymixin B and dissolved in distilled water (rALB14 was 0.33 mg/ml and rALB7 was 0.39 mg/ml).

Preparation of plasmas from BALB/c mice and Total IgE antibody assay

Five-week old male BALB/c mice (CLEA CO., LTD.,

Tokyo, Japan) were administrated 10 μg of rALB14 or rALB7 in 200 μl of aluminium hydroxide (LSL Co., LTD) by intraperitoneal injection. Fourteen days later, at the time of peak IgE antibody response, the plasmas were assayed by enzyme-linked immunosorbent assay (ELISA).

Preparation of spleen cells from BALB/c mice

Five-week old male BALB/c mice (CLEA) were sacrificed to collect spleen cells. The spleens were homogenized and washed with 2 ml of lysis buffer (1 mM NH_4HCO_3, 144 mM NH_4Cl) to remove red blood cells, then washed three times with 10 ml of PBS. The cells were finally adjusted to 2.0×10^6 cells/ml in 100 μl of RPMI-1640 medium (SIGMA) containing 5% fetal calf serum (FCS, SIGMA) and plated on 24-well plates (plates 1, 2, 3). On plates 1 and 2, rALB14, rALB7, and rDiAg were added as follows. rALB14; 20 μg/ml, 40 μg/ml, rALB7; 20 μg/ml, 40 μg/ml, rDiAg; 20 μg/ml, 40 μg/ml. On plate 2, 1 μg/ml of Con A (SIGMA) was added to each well. On plate 3, 20 μg/ml of rALB14 and 10 μg/ml of lipopolysacchalide (LPS, SIGMA) were added. The cells were cultured for 48 hrs at 37°C in a humidified 5% CO_2 atmosphere. Furthermore, on plate 4, spleen cells (5.0×10^6 cells/ml) were incubated. After 24-hrs incubation, non-adherent cells were harvested and recultured (2.0×10^6 cells/ml) with 10 μg/ml of rALB14 and 1 μg/ml of Con A for 48 hrs. Each well was incubated with 10 μl of 3-(4,5-Dimethyl-2-thazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Wako) (1 mg/200 μl) for 4 hrs at 37°C. The reaction was stopped with 90 μl of isopropanol containing 0.04 N HCl, and cell numbers were read at a wavelength of 570 nm.

T cell isolation from whole spleen cells

Spleen T cells were collected by nylon column, adjusted to 5.0×10^6 cells/ml, and incubated with 10 μg/ml of rALB14 in the presence or absence of 1 μg/ml of Con A for 48 hrs at 37°C in a humidified 5% CO_2 atmosphere. After incubation, cell numbers were measured by the MTT method.

IL-4 production assay by ELISA

The whole spleen cells were cultured with 10 μg/ml of rALB14 or 10 μg/ml of rALB7 for 72 hrs at 37°C in a humidified 5% CO_2 atmosphere. The supernatants of the cells were collected by centrifugation and the IL-4 production was measured by an IL-4 ELISA kit (Endogen Inc., MA).

Statistical analysis

All data obtained were analyzed with Student's *t*-test. $P < 0.001$ or $P < 0.005$ were considered to be significant.

Results

Sequence of polyprotein from A.lumbricoides

Fig. 1. (b) shows approximately 400-bp PCR products amplified by the primers ASPS1/ASPA2. This fragment was considered to be the linked fragment of the repeating unit as shown in Fig.1. (c), and after the transformation it was sequenced (Fig. 2). As shown, the full length of ALB was 399 bp, and we found four amino acids repeating, tetra-arginine, from 63 to 66. We supposed these arginines (Rs) to be the C-terminal region of one unit. Therefore rALB was designed as shown in Fig. 3. (a).

Design of rALB14 and rALB7

Fig. 3. (b) shows the design of rALB14 and rALB7. The amino acid sequence of ABA-1 shows the positions 13 to 68 and 69 to 124, giving 25% identity and 60.7% similarity between the sequence of the two putative domains[9], and this unique conformation is also observed in ALB. The positions 13 to 68 and 69 to 124 show 25.0% of identity, and 64.3% of similarity (Fig. 3. (c)). Therefore, to determine the minimum unit which induces non-specific IgE, we designed two types of rALB. rALB14 was the full length of ALB (133 residues) whereas rALB7 was the N-terminal half of ALB (67 residues) and is quite similar to the C-terminal half.

rALB analysis

Fig. 3. (d) shows the SDS-PAGE analysis. rALB14 was approximately 15 kDa and rALB7 was 7 kDa. These fragments were finally diluted in distilled water (rALB14 was 0.33 mg/ml, rALB7 was 0.39 mg /ml) and biological activity was measured.

Total IgE production in BALB/c mice

In *Ascaris* infection, the elevation of non-specific IgE has been investigated[1], and BALB/c mice (which are H-2^d haplotype) do not show the specific antibody to 15-kDa molecule thought to be ABA-1[12]. Therefore in the present study, we selected BALB/c mice to determine whether rALBs induce non-specific IgE. Fig. 4. (a) illustrates the total IgE production in BALB/c mice 14 days after inoculation with 10 μg/head of rALB14, rALB7 or rDiAg. The positive control used was rDiAg which

Fig. 2. DNA and amino acid sequences of ALB. The cloned ALB was sequenced by the dideoxy sequencing method. Resulted DNA sequence is shown. The amino acid sequence was deduced from ABA-1 sequence. The terminal tetra basic amino acid region of one unit is underlined.

induces total IgE in BALB/c mice (H. Tezuka, submitted to *Infect Immun*). rALB14 induced IgE more significantly than rALB7, however, it did not reach 400 ng/ml as was the case with rDiAg. Therefore, it was suggested that rALB7 N-terminal half can elevate only small amounts of total IgE, and that rALB14, the full length of ALB, is required to induce total IgE, though it is less effective than rDiAg.

Spleen cell proliferation of BALB/c mouse

Since IgE production is associated with immune cells such as B cells or T cells, we isolated spleen cells and observed the cell proliferations under stimulation of rALB14 or rALB7 (Table 1. (a)). When cells were stimulated by rALB14 or rALB7 alone, they did not show significant proliferation, but cells stimulated by both Con A and rALB14 (20 μg/ml, 40 μg/ml) or Con A and rALB7 (20 μg/ml) indicated significant proliferation. The ratio of rALB14 (20 μg/ml) to the control was 1.3 ± 0.002, and that of rALB7 was 1.2 ± 0.081. These responses are lower than the cells stimulated by Con A and rDiAg (20 μg/ml). The ratio to control of rDiAg was 1.5 ± 0.067. When the cells were stimulated by 40 μg/ml of rALB14 *or* rALB7, they showed different responses. When 40 μg/ml of rALB14 was added, the cell proliferation was still accelerated (the ratio to control was 1.5 ± 0.028). On the contrary, in response to rALB7, the cells showed cell death. The ratio to control of rALB7 was 0.8 ± 0.012 and was more significant than rDiAg, whose ratio to control was 1.4 ± 0.021.

To determine whether the biological activity of rALB14 is only accelerated in the presence of T cell mitogen, we also measured cell proliferation in the presence of LPS (B cell mitogen, macrophage stimulator). Table 1. (b) shows cell proliferation when stimulated by rALB14 (20 μg/ml) and LPS (10 μg/ml). Interestingly, the cells did not proliferate significantly when stimulated by rALB14 and LPS (the ratio to control was 0.9 ± 0.036). These results suggest that rALB14 might act on T cells rather than B cells or macrophages.

In addition, to clarify whether rALB acts on T cells to induce non-specific IgE production without co-stimulating B cells or macrophages, we examined the effect of rALB14 on non-adherent cells consisting of neither B cells nor macrophages. As shown in Table 1. (c), non-adherent cells showed significant proliferation when stimulated by rALB, Con A or rALB/Con A. Therefore it was suggested that rALB14 might act on non-adherent cells which do not contain B cells and macrophages.

T cell proliferation

Whole spleen cells proliferated under stimulation by rALB14 and Con A – known as T cell mitogen, but not under stimulation by LPS – known as B cell mitogen, and rALB14 alone caused proliferation of non-adherent cells. These observations suggested that rALB14 might have T cell mitogen-like activity. The proliferation of spleen T cells was measured (Table 1. (d)), and

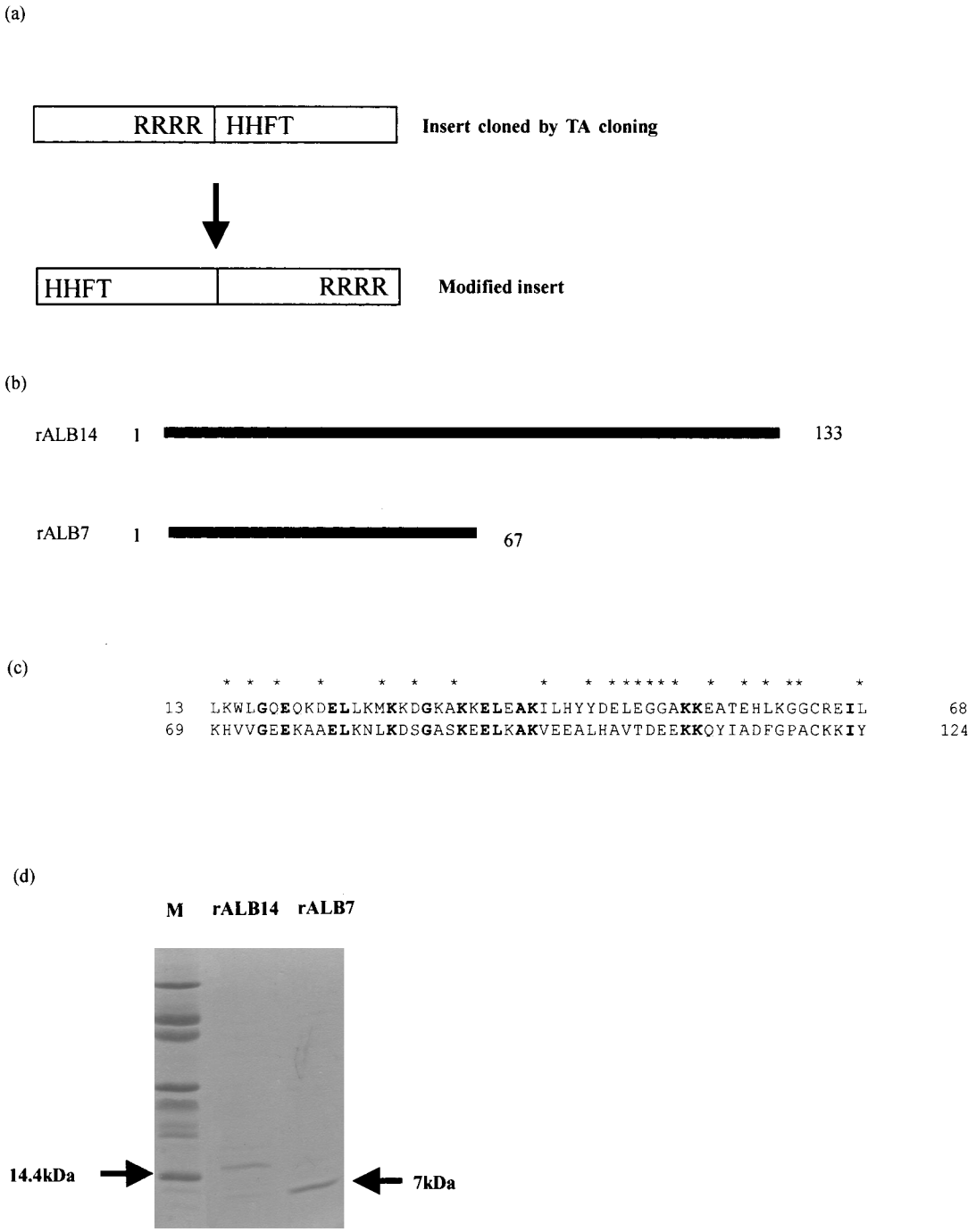

Fig. 3. (a) The design of recombinant ALB (rALB). The insert was amplified by PCR using primers ALP1S/ALP1AS and ALP2S/ALP2AS. Primer ALP1S (forward) was containing AflII site, primer ALP1AS (reverse) was containing BamHI site, primer ALP2S (forward) was containing NdeI site, ALP2AS (reverse) was containing AflII site. The digested PCR fragments were ligated and then the ligated DNA were amplified by PCR using the primers ALP2S /ALP1AS. (b) The designs of rALB14 and rALB7. rALB14 is a full-length amino acid sequence of ALB. rALB7 is a N-terminal half region of ALB. The numbers are the position of amino acid residues. (c) The comparison of the amino acid sequence of ALB from the position 13 to 68, and 69 to 124. Identical residues are shown in bold and similar residues are indicated by *. The homology of position 13 to 68 and 69 to 124 is 25.0%. The similarity of the same position is 64.3%. (d) SDS-PAGE stained with Coomassie blue showing rALB14 and rALB7. M: marker.

Table 1. (a) The ratio to control of spleen cell proliferation under the stimulation of rALB14, rALB7 or rDiAg in the presence or absence of Con A (1 μg/ml). control(Con A (-)); OD= 0.23±0.028, control (Con A(+)); OD= 0.31±0.004
*P< 0.001, **P< 0.005

			stimulant						
			rALB14		rALB7		rDiAg		
		control	20	40	20	40	20	40	(μg/ml)
Con A	-	1.0	1.1±0.048	1.1±0.031	1.1±0.081	1.1±0.017	**1.2±0.041	**1.4±0.050	
	+	1.0	*1.3±0.002	**1.5±0.028	**1.2±0.081	0.8±0.012	**1.5±0.067	**1.4±0.021	

(b) The ratio to control of B cell proliferation under the stimulation of rALB14 (20 μg/ml). control (LPS (-)); OD=0.09±0. 010, control (LPS(+)); OD=0.16±0.006
*P< 0.005

		control	rALB14
LPS	-	1.0	*1.1±0.033
	+	1.0	0.9±0.036

(c) The ratio to control of non-adherent cell proliferations under the stimulation of rALB14 (10 μg/ml) and/or Con A (1 μg/ml). control; OD=0.07±0.002
*P< 0.001

control	rALB14	Con A	rALB14/Con A
1.0	*1.6±0.056	*2.3±0.081	*2.8±0.089

(d) The ratio to control of T cell proliferation under the stimulation of rALB14 (10 μg/ml), Con A (1 μg/ml) or rALB14 and Con A. control; OD= 0.07±0.002,
*P< 0.001, **P< 0.005

	stimulant		
control	rALB14	Con A	rALB14/Con A
1.0	**1.6±0.039	*2.6±0.016	*4.5±0.072

The results are presented as the mean± S.D. in each group. (n=5)

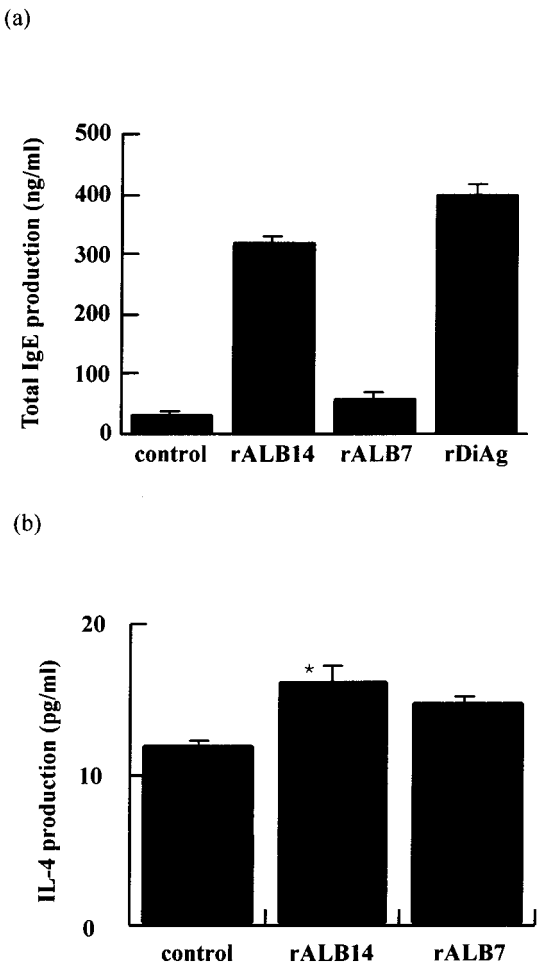

Fig. 4. (a) Total IgE production of BALB/c mice 14 days after the administration of rALB14, rALB7, or rDiAg. P< 0.001 (b) IL-4 production of whole spleen cells. *P< 0.005.

rALB14 had a slight degree of proliferation (the ratio to control was 1.6±0.039). Furthermore, rALB14/Con A stimulation showed synergism. Thus it was suggested that the biological activity of rALB14 might be involved in T cell proliferation.

IL-4 production of whole spleen cells

It is known that IL-4 production is strongly associated with IgE production[13–14]. Therefore, we measured the production of IL-4 in whole spleen cells (Fig. 4. (b)) and, cells stimulated by 10 μg/ml of rALB14 produced IL-4 slightly to a greater level than control and the case where 10 μg/ml of rALB7 was used ($P< 0.005$).

Discussion

In our present study, we first performed RT-PCR using the original primers based on the consensus region of ABA-1 and TBA-1. The fragments amplified with these primers were approximately 400 bp, and these results occurred because of the composition of ABA-1 with the repeating units[10].

After cloning, the DNA sequence of the ABA-1-like protein from *A. lumbricoides* (ALB) was identified, and the fragment whose carboxy terminal has tetra-basic amino acids residues---RRRR---was reconstructed to its original sequence conformation. By comparison with the sequences reported by McSharry *et al.*[15], ALB showed several point mutations in both nucleotides and amino acids. These point mutations might be caused by PCR errors. However, every ALB sequence point mutation agreed with at least *one* of the polymorphic monomer units of McSharrys', though the whole ALB sequence was different from the known sequences. Therefore our cloning sequence may be a novel one that does not contains PCR errors. These observations conclude that the sequence of polyprotein from *A. lumbricoides* is varied at both nucleotide and amino acid levels, as previously described by Xia *et al.*[16].

When compared with ABA-1 determined by Spence *et al.*[10] or TBA-1[17], ALB showed high homology in both nucleotide and in amino acid (data not shown). Therefore, the biological activities of ALB and ABA-1 or TBA-1 can be considered to be similar, and it is likely that their role in immune activity especially in the induction of an antibody such as IgE that is a characteristic of helminth infection, is similar as well.

Ascaris infection induces a total IgE that contains not only specific but also non-specific IgE antibodies, as indicated in other parasitic helminth infections[1]. It is known that an ABA-1-like protein induces parasite specific IgE antibodies in *A. lumbricoides* infection[18], however, the production of non-specific IgE antibodies observed at the beginning of the infection remains to be explained. Therefore, we constructed two types of recombinant polyprotein, rALB14 and rALB7, based on the conformation of ALB to determine the effect of ALB on the elevation of non-specific IgE. As it was indicated that the N-terminal and C-terminal halves show high homology in ALB, each half was considered to behave as one unit. Hence, we prepared full length (ALB14) and N-terminal half (ALB7) in the present study, treated BALB/c mice with rALB14 or rALB7 with a single dose, and measured the total IgE of plasmas by ELISA. After two weeks of inoculation with rALB14, the total IgE elevated significantly along with the rDiAg. This total IgE was considered to be non-specific IgE antibodies, since the inoculation of recombinants was challenged by the single dose and only a little specific IgE was induced (data not shown). We have therefore applied this observation to the situation of natural infection by parasites[1–2]. On the other hand, rALB7 slightly induced total IgE that we supposed to be non-specific, in the same manner as rALB14. Thus, the whole conformation of ALB seems to be involved in the induction of non-specific IgE production, and we also calculate that the production of non-specific IgE in *Ascaris* and *Toxocara* might be induced by NPA.

According to the previous study, rDiAg activated B cells and induced non-specific IgE production in mice (H. Tezuka, submitted to *Infect Immun*). To determine the effect of ALB on immune cells, we first stimulated spleen cells from naive BALB/c mice by rALB14 alone or rALB7 alone, and we found that both rALB14 and rALB7 induced poor proliferation. Furthermore in the presence of Con A, the cells demonstrated reasonable proliferation when stimulated by rALB14. This response can be explained by the reaction to Con A induced by rALB14.

On the other hand, rALB7 showed poor proliferation even in the presence of Con A, and high doses of rALB7 showed cell death when stimulated by Con A, although a high dose of rALB14 induced a more significant cell proliferation. It is reported that ES products from *Nector americanus* induce apoptosis in human T lymphocytes[19], and rALB7 matches the activity of ES products from *N. americanus*. These findings show that rALB14 stimulates spleen cell proliferation and that high doses of rALB7 induce cell death. On the contrary, when cells were treated with LPS - (known as a B cell

mitogen) – rALB14 did not induce cell proliferation. Therefore, it was deduced that rALB14 has the potential to activate T cells, but not B cells. It is reported that *Ascaris* body fluid (ABF) contains a B cell mitogen whereas ABA-1, similar to ALB, does not[20]. Additionally our results support the contention that the production of IgE in *Ascaris* infection in nude mice lacking T cells[21] does not occur.

IL-4 plays an important role in non-specific IgE production [13–14]. We suggest that ALB which is the NPA of *A. lumbricoides* might have the potential to activate T cells, so we measured the production of IL-4 to investigate its involvement in non-specific IgE production, and we observed the slight elevation of IL-4 after stimulating with rALB14 in whole spleen cells. The result suggested that rALB14 might activate T cells, and that rALB14 might be involved in non-specific IgE production. Moreover it concurs with the report that SJL mice which lack primary IL-4 production, show poor production of IgE in *Ascaris* infection[22]. Hence, in *Ascaris* infection, it seems to be important that T cells need to be activated by an ABA-1-like protein (NPA), for high levels of IgE production to occur. In fact, spleen cells without adherent cells showed significant proliferation when stimulated with a lower dose of rALB14 (10 μg/ml) than that used in the whole spleen cells (40 μg/ml).

The molecule which has T cell mitogen activity in the extracts of microfilariae of *Brugia malayi* has been identified[23]. Furthermore, it has been shown that NPA termed gp15/400 is contained in these extract from *B. malayi*[24]. This gp15/400 shows high homology to ABA-1, and rALB14 is similar to ABA-1, showing weak T cell mitogen activity. In addition, slight IL-4 production was confirmed in the whole spleen cells with rALB14, as well as in T cells with rALB14 (data not shown). From both these observations and previous studies, we can conclude that NPA from *Ascaris* might have the ability to induce the proliferation of T cells, and that these responses might be involved in non-specific IgE as well as in IL-4 production.

In summary, we determined the role of the ABA-1-like protein in *A. lumbricoides*, ALB. Unlike DiAg, ALB might be involved in activation of T cells, and might induce non-specific IgE production antibodies in BALB/c mice. Further study is required to understand more deeply, what other effects ALB has on T cells.

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