Passive immunisation against experimental salmonellosis in mice by orally administered hen egg-yolk antibodies specific for 14-kDa fimbriae of *Salmonella* Enteritidis

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Summary. Chickens were immunised with a preparation of purified 14-kDa fimbriae of *Salmonella* serotype Enteritidis (SEF 14) to raise egg-yolk antibodies for protection trials in mice against subsequent challenge-exposure with the homologous strain of Enteritidis. A pronounced specificity of egg-yolk antibodies against the 14-kDa fimbrial antigen was demonstrated by Western blotting analysis. Passive antibody protection was evaluated in a mouse model of experimental salmonellosis: 79 mice (CD 1 strain) were challenged orally with 2×10^{10} cfu of Enteritidis. Test mice treated with SEF-14 antibodies (titre = 128) had a survival rate of 77.8% compared to 32% survival in control mice fed normal egg-yolk antibodies (titre < 10) ($p < 0.01$). In-vitro adhesion of Enteritidis to mouse intestinal epithelial cells was reduced by anti-fimbrial antibodies. An indirect immunofluorescence method demonstrated the localisation of Enteritidis along the villous margins of the small intestine of control mice, whereas in test mice adherent bacteria were not detected. Results suggest that 14-kDa fimbriae may influence, enhance or contribute to the overall adhesive properties of Enteritidis and that egg-yolk antibodies directed against these fimbriae may have played a substantial role in protection, possibly by minimising bacterial colonisation and invasion during the early stages of infection.

Introduction

Salmonella serotype Enteritidis is the commonest serotype isolated in outbreaks of human salmonellosis in Europe and the USA.^{1,2} The global increase in the incidence of Enteritidis infection is attributed to the consumption of eggs and poultry that harbour the organism.^{3,4} In an attempt to curb the spread of the disease, studies by workers in this field to obtain a better understanding of the nature of the disease process have examined the mechanism of bacterial invasion in the host, the virulence determinants involved and, hence, the identification of suitable protective antigens for use in future vaccines and in the development of reliable diagnostic assays. Several types of surface structures expressed by Enteritidis have been identified and their possible roles in pathogenesis have been examined. Enteritidis has been shown to produce three types of fimbriae, referred to as SEF 14, SEF 21 and SEF 17 (or thin, aggregative pili) that are morphologically, biochemically and immunologically distinct.^{5–8} The 14-kDa fimbriae appear as fine, fibrillar structures, 3–5 nm in diameter, and are not associated with haemagglutinating activity.

A correlation between the presence of fimbriae and

bacterial virulence has been demonstrated in work with Enterobacteriaceae, such as *Salmonella* spp. and *Escherichia coli*, which produce a range of fimbrial types.^{9–12} An increase in virulence was attributed to the role of fimbriae in facilitating bacterial adherence to mucosal epithelium. Conversely, decreased virulence was due either to loss of fimbriation or to the inhibitory action of anti-fimbrial antibodies on bacterial adhesion.^{13,14} In another study, bacterial virulence was reduced by passive immunisation with chicken egg-yolk anti-fimbrial antibodies.¹⁵ The use of anti-K88, -K99 and -987P fimbrial antibodies, derived from egg yolks of immunised chickens, conferred protection in piglets against experimental oral infection with enterotoxigenic *E. coli*. The result of that experiment provided further evidence for a role of fimbriae as a virulence determinant and indicated that the inhibitory action of antibodies directed against these bacterial structures could reduce bacterial pathogenicity, probably by inhibition of bacterial colonisation and invasion.

Passive protection by egg-yolk antibodies may serve as a tool for the control of intestinal colonisation by salmonellae. In the continued spread of the bacteria, the concept of passive anti-fimbrial antibody protection may have far-reaching importance and implications for the control of salmonellosis in poultry; hence, it may also help disrupt the transmission of

disease to man. Chicken egg-yolk antibodies are cheaper to produce in large volume than antibodies derived from serum, colostrum or by monoclonal techniques and their efficacy has been reported.^{15, 16-19}

The role of SEF 14 in bacterial adhesion and pathogenicity has not been established. This study was undertaken to gain insights into the possible role of SEF 14 in virulence, their value as immunising antigens and the potential for protection of egg-yolk antibodies directed against these fimbriae *in vitro* and *in vivo* in a mouse model of infection, and possibly extending thereby their efficacy to other species in the form of oral antibody preparations suitable for use in animals or man.

Materials and methods

Bacteria and growth conditions

Salmonella Enteritidis type strain ATCC 13076 was obtained from Dr T. Ezaki, College of Medicine, University of Gifu, Gifu City, Japan. It was grown in static colonisation factor broth (CFB) at 37°C for 120 h. After incubation, bacteria were harvested by centrifugation (12000 *g* for 20 min) at 4°C, washed twice in PBS and the final bacterial pellet was suspended in 0.15 M ethanolamine buffer (pH 10.5) for preparative extraction of 14-kDa fimbriae. For challenge exposure of mice, Enteritidis was grown overnight in brain heart infusion (BHI) broth at 37°C with shaking. The bacterial pellet obtained after centrifugation as above was suspended in PBS and thereafter injected into mice.

Purification of 14-kDa fimbriae

The procedures for purification of SEF 14 followed those described previously.⁷ The purity of fimbrial preparations was analysed by SDS-PAGE in acrylamide 15% w/v gel with low mol. wt markers (BioRad Laboratories, Richmond, CA, USA) and computer densitometric scanning of gel profiles (Geru Hakusi, Mitani Corp., Fukui, Japan). Fimbrial morphology was also examined by transmission electronmicroscopy (H-300, Hitachi, Tokyo, Japan) with negative staining. The concentration of fimbriae was measured by a protein-assay system (BioRad) with bovine serum albumin as reference protein; the fimbrial preparation was later used for the immunisation of chickens.

Immunisation of chickens

Five-month-old White Leghorn chickens (strain Hyline W36) were immunised with fimbriae for the development of egg-yolk fimbria-specific antibodies. Fimbrial extract, containing 0.5 mg of fimbrial antigen emulsified in Freund's complete adjuvant (1:1) was injected i.m. in the breast muscles of chickens. Six weeks after the first injection, chickens received booster injections of the same dosage by the same

route of administration and this was repeated 2 weeks later. A single day's eggs were collected 2 weeks after the final injection and were processed.

Haemagglutination

The haemagglutinating activity of SEF-14 fimbriae was assayed on glass slides with heparinised blood from chicken, rabbit, mouse and guinea-pig; erythrocyte suspensions 3% v/v in PBS were prepared. Equal volumes of fimbrial suspensions in PBS and erythrocyte 3% suspensions were mixed on glass slides at room temperature for 5 min.

Extraction of antibodies from egg yolks

Yolks obtained from the eggs of 10 immunised and five non-immunised chickens were processed separately. About 20-30 egg yolks were separated carefully from egg whites, pooled and mixed in a 1-L beaker. After moderate stirring with a mixer, egg yolks were diluted with an equal volume of PBS to which 2 volumes of chloroform were then added, and incubated at 25°C for 30 min. The mixture was centrifuged (2000 *g* for 10 min) at 4°C and the supernate, i.e., the water-soluble immunoglobulin-rich fraction, was separated and freeze-dried in a Labconco freeze-drying machine (Labconco LL-12, Labconco Corp., Kansas, MO, USA). The antibody powder was stored in a desiccator at room temperature until used. The specificity of egg-yolk antibodies was determined by Ouchterlony's immunodiffusion test²⁰ and by Western blotting.²¹

Titration of antibodies

The antibody titre against the challenge-exposure strain was determined by a microtitration plate agglutination method. Inactivated whole Enteritidis bacteria were used as antigen in the agglutination test. Bacteria were suspended in PBS after culture for 18 h in BHI broth at 37°C, washed twice and resuspended in formol 0.5% PBS at 37°C for 18 h to inactivate the bacteria. Samples of bacterial suspension were added to PBS to a desired optical density (0.42 at 620 nm). The adjusted bacterial suspension was used as antigen; 0.05 ml was added to equal volumes of two-fold serial dilutions of a 10% antibody solution prepared by reconstituting freeze-dried antibody powder (0.1 g dry weight) in 0.9 ml PBS. After overnight incubation at 25°C, the titre was defined as the highest dilution of antibody powder showing agglutination.

Western blotting

Separated antigens were transferred from an SDS-PAGE gel (fig. 1, lane A, 2) to a nitrocellulose membrane by electroblotting at 170 mA for 1 h in a tris-glycine-methanol transfer buffer. The blotted nitrocellulose membrane was washed in PBS-Tween

and cut into strips before immersion in BSA 3% w/v for 18 h at room temperature. After three 5-min washes in PBS-Tween, antigens on the membrane were treated with absorbed chicken egg-yolk SEF-14 antibody solution for 2 h at 37°C. A 10% v/v antibody solution was made initially and diluted (1 in 100) to react with the membrane proteins. Binding of the absorbed chicken egg-yolk SEF-14 antibody to fimbrial antigens was detected by incubation with peroxidase-conjugated anti-chicken IgG (diluted 1 in 500) for 1 h at 37°C. After three washes in PBS-Tween, the reaction was revealed by the use of *o*-phenylenediamine dihydrochloride and hydrogen peroxide as substrate.

Absorption of chicken egg-yolk antibody

Preparations of monospecific egg-yolk antibody solution required absorption with homologous non-fimbriate cultures of *Enteritidis*. Smooth colonies, taken from blood agar, were inoculated into BHI broth and incubated overnight at 18°C, a temperature at which fimbriae are not formed. Bacteria were harvested and suspended in PBS. Equal volumes (*c.* 1 ml) of bacterial suspension and egg-yolk antibody solution (titre 128) were mixed and incubated for 2 h at 50°C. After centrifugation, the clear supernate was collected and the absorption process repeated. The absorbed egg-yolk antibody solution was tested for monospecific reaction against homologous antigen by Western blotting.

Challenge exposure of mice to Enteritidis and oral administration of antibodies

A total of 79 mice, 5–7 weeks-old, *c.* 25 g in weight, was randomly distributed into three groups. Group I (control): each of 25 mice was inoculated orally with 0.2 ml of bacterial suspension (2×10^{10} cfu) with pre-treatment of 0.2 ml of normal egg-yolk antibody solution (0.1 g of normal egg-yolk powder was reconstituted in 0.9 ml of PBS, titre < 10). Group II: each of 27 mice received 0.2 ml of bacterial suspension with pre-treatment with 0.2 ml of SEF-14 egg-yolk antibody solution (0.1 g of SEF-14 antibody powder reconstituted in 1.9 ml of PBS, titre 32). Group III: each of 27 mice received 0.2 ml of bacterial suspension with pre-treatment of 0.2 ml of egg-yolk antibody solution (0.1 g of SEF-14 antibody powder reconstituted in 0.4 ml of PBS, titre 128). Pre-treatments with corresponding antibody solutions were given as single oral doses *c.* 15 min before bacterial challenge. The bacterial inoculum was administered by gastric intubation with a blunt needle attached to a syringe. About 3 h after inoculation, the same antibody solutions were administered orally (0.2 ml/mouse) by the same method and repeated three times per day for 3 days to test their efficacy against the experimental salmonella infection in mice. Mice were observed daily for 1 week for clinical signs of infection and survival

(%) of mice was calculated. The mice were grouped in metal cages placed in a well-ventilated isolation room and had free access to food and drinking water. Mice surviving at the end of the experiments were killed by a quick cervical dislocation technique.

In-vitro adherence of Enteritidis to enterocytes and inhibition by SEF-14 antibodies

Mouse small intestine epithelial cells. A mouse was killed and the small intestine was exposed immediately and excised. Mucus and food residue were removed by repeated washing with PBS through a syringe attached to the proximal end of the intestine. The distal end of the intestine was ligated and the intestine was filled with EDTA buffer (8 mM KH_2PO_4 , 6.5 mM KCl and 10 mM EDTA, pH 6.8). The proximal end of the intestine was ligated and allowed to incubate at room temperature for 15 min on a shaker. EDTA buffer solution was drained and the intestine was filled with PBS. The epithelial cells were detached from the villi by gently massaging the intestine between the fingers and collected by draining into a sterile petri dish. The isolated cells were washed twice in PBS and dispersed into a single cell suspension by repeated passage through a 24-gauge needle.

Mouse small intestine epithelial cell adherence. Equal volumes (1 ml) of epithelial cell (10^7 /ml) and bacterial suspensions (10^9 cfu/ml) were incubated at 37°C for 30 min. The succeeding procedures in the adhesion assay were performed as described before.¹⁵ Bacterial attachment to epithelial cells was inhibited with anti-fimbrial antibody preparations by pre-incubating the mixture of 1 ml of bacteria and 1 ml of egg-yolk antibody solution (titre 128) for 15 min, adding 1 ml of epithelial cell suspension, followed by incubation at 37°C for 30 min. A similar adhesion-inhibition procedure was performed with rabbit antiserum (titre 320) as the pre-incubating antibody preparation. The number of bacteria attached to 25 epithelial cells was determined, and the average number of bacterial cells attached to each epithelial cell was calculated.

Indirect immunofluorescence test

The small intestines of mice were examined after oral infection with *Enteritidis* with or without antibody pre-treatment. The tissue specimens were obtained from a duodenum, jejunum and ileum of the small intestine of: (1) a mouse pre-treated with SEF-14 egg-yolk antibodies and challenge-exposed with *Enteritidis*; (2) a mouse challenge-exposed with *Enteritidis* only; (3) a control mouse that received neither antibody nor bacteria; and (4) a control mouse with normal egg-yolk antibody and challenge-exposed with *Enteritidis*. A section (*c.* 2 cm) of intestine was excised from each mouse and immersed in cold *n*-hexane at -80°C overnight. Tissue was supported in gelatin specimen-embedding compound Bright Cryo-M-Bed (Bright Instruments, Cambridge) and

cut transversely into 8- μ m thin sections on a Bright Cryostat model OTF/AS-600 microtome at -20°C . Sections were then mounted on slides, blow-dried, fixed in acetone at -20°C for 10 min and stained. Anti-SEF-14 rabbit serum was used as the first serum and fluorescein isothiocyanate-conjugated anti-rabbit IgG (Cappel, Organon Teknika Co., PA, USA) was used as the second serum in reactions with all specimens.

Production of antisera

Antisera were raised in rabbits against SEF-14 fimbriae purified by the modified Muller method. An initial s.c. injection was given in the back with 30 μg of antigen emulsified in Freund's complete adjuvant (1 to 1). Two weeks after the first injection, a booster was given s.c. with antigen emulsified in Freund's incomplete adjuvant. The rabbits were bled 2 weeks after the booster injection.

Statistical analysis

The statistical significance of differences in survival rates between the treated and control groups was assessed by Fisher's exact test, and the mean differences in bacterial adhesion *in vitro* between the control and pre-incubated groups was assessed by the Tukey test.

Results

Purification of SEF 14

A straightforward purification procedure for SEF 14 was followed as described previously,⁷ with slight modification. The 14-kDa fimbrial antigen obtained by this method resulted in a preparation of high purity.

Fig. 1. Analysis of purified Enteritidis SEF 14 showing: A, Coomassie-stained SDS-PAGE; lane 1, mol.-wt markers; 2, fimbriae and surface materials; 3, purified SEF 14 ($\leftarrow$); and B, Western blot analysis; lane 4, same sample as in SDS-PAGE, lane 2, electrophoresed and exposed to absorbed immune egg-yolk antibodies raised against SEF 14 (single band indicated by arrow).

SDS-PAGE analysis of this preparation demonstrated the purification of 14-kDa fimbriae to homogeneity as assessed by Coomassie R-staining, with minor contaminating bands seen on the gel profile (fig. 1A, lane 2). Additional treatment of the same fimbrial sample with SDS 0.2% further removed impurities and gave a more purified SEF-14 protein (fig. 1A, lane 3). Computer densitometric scanning analysis of the protein profile after SDS-PAGE calculated the purity of SEF 14 at 96.4%. Electronmicroscopy of SEF 14 recognised a fimbrial morphology, as previously described. The purified 14-kDa fimbriae were non-haemagglutinating.

Antibody titres of freeze-dried powder to SEF 14

The final antibody freeze-dried powder was tested for immunoreactivity against SEF 14 by a microplate agglutination method, immunodiffusion technique and immunoblotting. The titre of the antibody solution was 64 (1 in 10 dilution of antibody powder) and correlated with titres obtained from the serum and water-soluble fraction of egg yolks from immunised hens. The titre of a 1 in 10 dilution of antibody powder produced from egg yolks of non-immunised hens was < 10 against the antigens used in the assay. Ouchterlony immunodiffusion technique demonstrated a pronounced precipitation line between egg-yolk antibody and purified SEF 14, indicating a specificity of egg-yolk antibodies to the target antigen (data not shown). Western blotting of specific egg-yolk antibodies raised against SEF 14 with electrophoretically transferred nitrocellulose membrane-bound surface proteins of Enteritidis revealed a single immunoreactive band at the 14-kDa level (fig. 1B, lane 4).

Clinical evaluation of mice after challenge exposure with Enteritidis and passive immunisation with antibody powder

The clinical responses of mice after challenge and subsequent treatment with antibodies are shown in table I. Mice in group III (high-antibody group: titre 128) showed the highest survival rate, with a 77.8% recovery rate from Enteritidis infection. Mice in group II (low-antibody group: titre 32) had a 59.3% survival, whereas control mice administered egg-yolk antibodies from non-immunised chickens had the lowest survival rate at 32%. There was a significant difference in the survival rates between the high-antibody group and the control group ($p < 0.01$). The infected mice manifested the following clinical signs: lethargy, anorexia, ruffling and dullness of the hair coat, swelling of the eyelids, congestion of the conjunctivae with watery discharge and death. No overt diarrhoeal disease was observed. Necropsy findings from carcasses of mice that died from infection revealed inflammation of the small intestines. Large intestines contained formed ingesta and faeces. No remarkable gross abnormalities were seen on liver, spleen and

Table I. Clinical response of mice challenge-exposed with Enteritidis and treated with anti-SEF-14 antibodies

Mouse group*	Number tested	Antibody titre	Challenge dose	Number of mice harbouring Enteritidis in internal organs/total	Number of mouse survivors/total on day			Survival (%)
					1	3	7	
I	25	< 10	2×10^{10}	25/25	17/25	10/25	8/25	32
II	27	32	2×10^{10}	17/27†	21/27	19/27	16/27	59.3%†
III	27	128	2×10^{10}	10/27‡	24/27	21/27	21/27	77.8%‡

* Group I, control mice were treated with egg-yolk antibodies from non-immunised chickens (titre < 10). Mice in groups II and III were treated with egg-yolk antibodies from chickens immunised with purified SEF-14 protein at antibody titres of 32 and 128, respectively.

† $p < 0.05$.

‡ $p < 0.01$.

Table II. Effect of anti-SEF-14 antibody on in-vitro adhesion of Enteritidis to mouse small intestinal cells

Bacterial suspension	Number of bacteria attached per epithelial cell
Control (not pre-incubated with antibodies before assay)	22.5 SD 7.6
Pre-incubated with egg-yolk SEF-14 antibody (titre 128)	10.0 SD 6.7*
Pre-incubated with rabbit antiserum (titre 320)	5.0 SD 2.5*

* $p < 0.01$.

Fig. 2. In-vitro adhesion of Enteritidis to mouse small-intestinal epithelial cells, showing: **A**, epithelial cell with adherent bacteria on the brush border, lateral and basal surfaces; **B**, epithelial cells with few adherent bacteria (antibody-pre-incubated Enteritidis) ($\times 900$).

kidney. Infecting bacteria were isolated from swab specimens of liver, spleen, kidney and intestines of dead mice, suggesting a septicaemic form of infection. Some mice, which survived the experimental challenge, harboured the infecting bacteria in liver and spleen, as indicated by positive cultures from swab specimens taken when the mice were killed.

In-vitro adhesion-inhibition assay

Enteritidis adhered to isolated murine small intestinal epithelial cells to varying degrees. It was observed to attach to the brush border, lateral and basal borders of epithelial cells. The average number of adherent bacteria per epithelial cell was 22.5 SD 7.6

Fig. 3. Indirect-immunofluorescence test. **A**, ileal section of small intestine from mouse pre-treated with egg-yolk SEF-14 antibodies and challenged-exposed with Enteritidis. Note the absence of clear fluorescence along the villous margin. **B**, ileal section of small intestine from mouse challenged-exposed with Enteritidis with pre-treatment with normal egg-yolk antibodies, showing fluorescence along the villous margin.

(table II). The attachment of bacteria to epithelial cells was reduced by homologous anti-fimbrial antibody solution either from serum or egg-yolk antibody solution. Fig. 2 shows the in-vitro adhesion of Enteritidis to small intestinal epithelial cells from a mouse. The control epithelial cells (mixed with Enteritidis not pre-incubated with antibody) showed adherent bacteria on their surfaces, whereas epithelial cells mixed with antibody-pre-incubated Enteritidis showed a relatively low number of bacteria attached to their surfaces.

Fluorescent antibody test

Specimens of intestinal tissue from mice treated with anti-SEF-14 antibodies (titre 128) and challenged with Enteritidis did not show fluorescence along the villous margins of the intestinal epithelium by indirect immunofluorescent microscopy (fig. 3). A similar result was obtained with normal intestinal tissue from control mice that had received neither antibody nor bacteria (data not shown). In contrast, intestinal specimens from mice challenged with Enteritidis and not pre-treated with antibodies, or from mice pre-

treated with normal egg-yolk antibodies, showed positive fluorescence along the villous margin of the intestinal epithelium.

Discussion

This study attempted to provide preliminary data on the use of chicken egg-yolk immunoglobulins raised against the 14-kDa fimbriae of Enteritidis for the control of experimental salmonellosis in mice. Passive protection was achieved with orally administered anti-SEF-14 antibodies, resulting in a 77.8% recovery rate from Enteritidis infection in mice treated with a high level of antibody (titre 128) and 59.3% in mice treated with a lower level of antibody (titre 32). Control mice that were fed normal egg-yolk antibodies, from the eggs of non-immunised chickens, had the least number of survivors, with a recovery rate of 32%. These findings are in agreement with our previous findings in passive antibody immunisation studies in other animal species, in which the protection achieved was dose-dependent.^{15,19} The anti-fimbrial agglutinin titre of egg-yolk antibody solution from non-immunised hens was negligible and the protective capacity was considerably lower. On the other hand, the egg-yolk antibodies from immunised hens offered a degree of acquired resistance to disease in mice, suggesting that protection may have been conferred by fimbrial antibodies present in the preparation.

The mechanism by which the anti-SEF-14 antibodies might reduce the pathogenicity of Enteritidis is not known but may be inferred from our results. It is widely accepted that bacterial adhesion to intestinal epithelia is mediated by fimbriae and interfering with the adhesive properties of these structures with fimbrial antibodies may reduce bacterial virulence. In the case of Enteritidis, various fimbrial antigens (SEF 14, 21 and 17) have been identified and implicated as having a possible role in bacterial attachment and colonisation. The results of these protection trials suggest that the efficacy resulting from the use of fimbrial antibodies is probably due to inhibition of the biological activities of fimbriae that might reduce the overall virulence properties of the bacteria. The 14-kDa fimbriae, among other fimbrial types, may mediate or enhance attachment of the bacteria to the intestinal epithelium. In-vitro adhesion assays demonstrated that Enteritidis adhered to intestinal epithelial cells. Moreover, pre-incubation of the bacteria with anti-SEF-14 antibodies in a parallel adhesion assay revealed relatively poor bacterial adherence. Although the number sometimes varied, the results overall of the adhesion-inhibition assays suggested a contributory role for 14-kDa fimbriae in bacterial attachment. The utilisation of fimbrial antibodies in the assays with specificity against a single type of fimbrial antigen for in-vitro inhibition of bacterial adhesion might be complicated by the variety of fimbrial antigenic types present in Enteritidis and the possible mediation of

adhesion by surface antigens other than fimbriae. A more convincing role for anti-SEF-14 antibodies was demonstrated in the indirect immunofluorescence test, in which correlation between the presence of antibodies and the inhibition of bacterial adhesion was observed. In mice pre-treated with fimbrial antibodies (at a titre of 128) and challenge-exposed with Enteritidis, bacterial colonisation of the small intestines was minimised, as can be inferred from the poor immunofluorescence observed along the villous epithelium. In contrast, intestinal specimens from control mice pre-treated with normal egg-yolk antibodies had a more pronounced immunofluorescence along the villous margins of the intestines, indicating the presence of adherent bacteria.

The clinical response of mice to Enteritidis infection was characterised by progressive development of disease and rapidly fatal infection. The CD 1 mice used in this study were infected with Enteritidis at a high challenge dose of 2×10^{10} cfu/mouse by the oral route. The infected mice did not manifest any overt diarrhoeal disease throughout the observation period, suggesting that the salmonella infection was extra-intestinal or systemic in nature. The systemic infection probably started with penetration of the intestinal epithelium by the bacteria and their subsequent dissemination throughout the reticulo-endothelial system, where they localised and multiplied. In mice that died of infection, either in the control or antibody treated groups, the challenge strain was isolated from the liver, spleen, kidney and small intestine. On the other hand, of the mice that survived in the antibody-treatment group and were killed at the end of the trial, only a few harboured the bacteria in the various organs. Therefore, the prophylactic value of anti-SEF-14 antibodies lies in their ability to inhibit the initial attachment of Enteritidis to membrane surfaces and subsequent tissue invasion. Once bacterial invasion and dissemination have begun, fimbrial antibodies would have little use or influence in the course of the disease and protection would depend on the involvement of the cell-mediated immune response or the presence of circulating antibodies.

The data presented here suggest that the 14-kDa fimbriae of Enteritidis may serve as a protective antigen; the antibodies raised against the fimbriae afforded protection against salmonellosis in this animal model. However, a few observations were noted when 14-kDa fimbriae were used as immunogens. The production of 14-kDa fimbriae by Enteritidis is variable and an attempt to produce the fimbrial antigens on a larger scale and reproducibly for inclusion in purified fimbriae or enriched fimbrial antigens may be difficult. The antibody response stimulated in chickens by repeated immunisation of 14-kDa fimbriae elicited a relatively low agglutinin titre. This finding agreed with that in an earlier investigation,²² that a low level of agglutinins is stimulated in chicken sera in response to infection by salmonella food-poisoning serotypes. Other factors that might have influenced the level of

antibody response include the breed and age of chickens used for immunisation and the sensitivity of the titration method used in this study. The immunological response to infection by salmonellae is greater in mature chickens²³ and the breed of chicken may also affect the response; for example, White Leghorn chickens produce lower agglutinating antibody titres to Gallinarum than do Rhode Island Reds.²⁴ In this study, young White Leghorn chickens were immunised before their egg-laying age and this too may have influenced the low level of antibody response. Nevertheless, there was a pronounced sero-specificity against the purified SEF-14 antigens with polyclonal antiserum prepared in rabbits and chickens as well as with the egg-yolk antibodies, as indicated by the strong

immunoprecipitation of purified fimbriae detected by the Ouchterlony immunodiffusion techniques (data not shown). Moreover, Western blot experiments with SEF-14 and egg-yolk antibodies demonstrated a positive result, indicating the specificity of the fimbrial antibodies. The delivery of fimbrial antigens at low dose during vaccination may have increased affinity maturation and biased isotype switching to optimise the quality of antibody generated.²⁵

The beneficial effects observed with the use of chicken egg-yolk anti-SEF-14 antibodies against experimentally induced Enteritidis infection in mice in this study may encourage further exploration of their prophylactic value for the control of salmonellosis in other animal species.

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