ELSEVIER

European Journal of Pharmacology 336 (1997) 203–209

ejp

Effect of a monoclonal antibody against interleukin-4 on the induction of oral tolerance in mice

Shin Yoshino [a,*], Motoyasu Ohsawa [b]

[a] *Department of Microbiology, Saga Medical School, 5-1-1 Nabeshima, Saga 849, Japan*
[b] *Department of Environmental Toxicology, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-09, Japan*

Received 22 May 1997; revised 31 July 1997; accepted 5 August 1997

Abstract

The present study was undertaken to investigate the effect of a monoclonal antibody against interleukin-4 on the induction of oral tolerance. Oral tolerance was induced by feeding mice with low and high doses (0.1, 1 and 10 mg) of hen egg lysozyme once a day for 5 days before immunization with the antigen. An anti-interleukin-4 monoclonal antibody was i.p. injected 30 min before each oral administration of hen egg lysozyme. The results showed that the oral administration of hen egg lysozyme suppressed immune responses to the antigen including delayed type hypersensitivity, production of both isotypes of immunoglobulin (Ig) G1 and IgG2a antibodies and proliferation of lymph node cells in a dose-dependent manner. The suppression of these responses by the oral antigen was associated with a marked reduction of interferon-γ secretion and a moderate decrease in interleukin-4 production by lymphoid cells. The treatment with the anti-interleukin-4 monoclonal antibody blocked dose-dependently the suppression of the delayed type hypersensitivity response to hen egg lysozyme, anti-hen egg lysozyme IgG2a antibody production and interferon-γ secretion. In contrast, the anti-interleukin-4 antibody facilitated the suppression of anti-hen egg lysozyme IgG1 antibody production and interleukin-4 secretion. Thus, the neutralization of interleukin-4 by anti-interleukin-4 antibodies appears to be effective in modulating the induction of oral tolerance. © 1997 Elsevier Science B.V.

Keywords: Oral tolerance; Interleukin-4; Interferon-γ

1. Introduction

Oral administration of an antigen induces peripheral immune tolerance to the antigen termed oral tolerance (Mowat, 1987; Weiner et al., 1994). It is thought to contribute to the prevention of food hypersensitivity (Mowat, 1993). Recent studies also indicated that feeding pathogenic antigens was effective in suppressing a variety of autoimmune disorders (Nagler-Anderson et al., 1986; Higgins and Weiner, 1988; Nussenblatt et al., 1990). Although the exact mechanism of induction of oral tolerance still remains obscure, possibilities include deletion (Chen et al., 1995) and anergy (Whitacre et al., 1991) of antigen-specific lymphocytes and suppression by inhibitory cytokines including transforming growth factor-β and interleukin-4 secreted from regulatory T-cells (Lider et al., 1989; Chen et al., 1994). In particular, it has been shown in mice that low doses (less than 1 mg) of oral antigens favor cytokine-mediated active suppression and high doses (more than 5 mg) favor anergy (Friedman and Weiner, 1994).

Interleukin-4 was originally identified as a mediator that induces the proliferation of murine B-cells (Howard et al., 1982). Cellular analysis revealed that the source of interleukin-4 was Th2 cells, a subset of $CD4^+$ T-cells (Mosmann and Coffman, 1989). Interleukin-4 has been demonstrated to be crucial in the production of IgG1 and IgE antibodies, while this cytokine inhibits the production of IgG2a antibodies (Isakson et al., 1982). It has also been shown that pronounced production of interleukin-4 is observed in mice fed antigens (Khoury et al., 1992; Fishman-Lobell et al., 1994). These findings suggest that the neutralization of interleukin-4 by anti-interleukin-4 antibodies may modulate oral tolerance. In the present study, we show that treatment of mice with a monoclonal antibody against interleukin-4 was effective in blocking the suppression by the oral administration of hen egg lysozyme of the delayed type hypersensitivity response to the anti-

* Corresponding author. Tel.: (81-952) 342-246; Fax: (81-952) 333-116; e-mail: yoshins@post.saga-med.ac.jp

gen, the production of anti-hen egg lysozyme IgG2a antibodies and the secretion of interferon-γ, while the suppression by the oral antigen of anti-hen egg lysozyme IgG1 antibody production and interleukin-4 secretion was facilitated in animals treated with the anti-interleukin-4 monoclonal antibody.

2. Materials and methods

2.1. Animals

Male DBA/1J mice, 8 to 9 weeks of age, were used in all experiments. The mice were bred in the animal breeding unit of Saga Medical School (Saga, Japan). They were maintained in a temperature- and light-controlled environment with free access to standard rodent chow and water.

2.2. Immunization

Mice were immunized s.c. in the tail base with 100 μg of hen egg lysozyme (Sigma, St. Louis, MO, USA) dissolved in 50 μl of 0.9% NaCl and emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA) (day 0).

2.3. Administration of hen egg lysozyme

Mice were fed 0.1, 1 and 10 mg of hen egg lysozyme dissolved in 0.5 ml of phosphate-buffered saline (PBS) through a syringe fitted with an 18 G ballpoint needle on days −5, −4, −3, −2 and −1 before immunization with hen egg lysozyme. 0.5 ml of PBS and 10 mg of keyhole limpet hemocyanin (Calbiochem, La Jolla, CA, USA) dissolved in 0.5 ml of PBS were given similarly for 5 days as controls.

2.4. Treatment with an anti-interleukin-4 monoclonal antibody

The cell line for a rat IgG1 monoclonal antibody against murine interleukin-4 was kindly provided by the Department of Immunology, Saga Medical School. The anti-interleukin-4 monoclonal antibody was precipitated by ammonium sulphate from ascitic fluid of SCID mice inoculated with the cells and purified using a protein G Sepharose 4FF column (Pharmacia Biotec, Tokyo, Japan), dialyzed with PBS and filtered. The protein content was quantified by absorbance measurement at 280 nm. The preparation and characterization of the anti-interleukin-4 monoclonal antibody have been described previously (Ohara and Paul, 1985). Varying doses of the anti-interleukin-4 monoclonal antibody dissolved in 0.5 ml of PBS were injected i.p. 30 min before each hen egg lysozyme feeding. As treatment controls, 0.5 ml of PBS only and 0.5 ml of PBS containing normal rat serum IgG purified as described above were given to mice.

2.5. Measurement of delayed type hypersensitivity

On day 12 after immunization, 10 μg of hen egg lysozyme dissolved in 20 μl of PBS was injected s.c. into the right footpad. As a vehicle control, 20 μl of PBS was injected into the left footpad. The thickness of the right and left footpad were measured using dial gauge calipers calibrated with 0.01 mm graduations (Ozaki MFG, Tokyo, Japan) immediately before and 24 h after the challenge injection. The increase in left footpad thickness was subtracted from the increase in right footpad thickness to give the value due to the specific response to the antigen. In unsensitized mice, responses to hen egg lysozyme and PBS were essentially equivalent.

2.6. Measurement of anti-hen egg lysozyme antibodies

Blood was collected on day 21 after immunization and sera were heat inactivated at 56°C for 30 min. Anti-hen egg lysozyme IgG1 and IgG2a antibodies were measured using an enzyme-linked immunosorbent assay (ELISA) (Enavall and Perlmann, 1972). In brief, 96-well flat-bottomed microtiter plates were incubated with 100 μl/well of hen egg lysozyme (100 μg/ml) at 37°C for 1 h and washed three times with PBS containing 0.05% Tween 20. The wells were then blocked by incubation with 100 μl of PBS containing 1% ovalbumin (Sigma) at 37°C for 1 h. After washing, the plates were incubated with 100 μl of a 1:10 000 dilution of each serum sample at 37°C for 30 min. The plates were washed and 100 μl/well of a 1:1000 dilution of rat anti-mouse IgG1 or IgG2a labeled with alkaline phosphatase (Pharmingen, San Diego, CA, USA) was added and incubated at 37°C for 1 h. After washing, 100 μl of 3 mM of *p*-nitrophenylphosphate (Bio-Rad, Hercules, CA, USA) was added per well and the plates were incubated in the dark at room temperature for 15 min. The absorbance was then measured at 405 nm in a Titertec Multiscan spectrophotometer (EFLAB, Helsinki, Finland). The results were expressed as absorbance units at O.D. 405 ± standard errors of the mean (S.E.M.).

2.7. Proliferation assay

Mice were killed 14 days after immunization and single cell suspensions were prepared from their inguinal lymph nodes. A total of 5×10^5 cells, in 100 μl of RPMI 1640 (Flow Laboratories, Mclean, VA, USA) containing 1 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol and 1% heat-inactivated autologous mouse serum were added to each microwell followed by the addition of 100 μl of 50 μg/ml hen egg lysozyme. The cells were cultured for 72 h. Each well was

pulsed with 0.5 μCi of tritiated thymidine, and the cells were cultured for another 16 h. The cultures were harvested onto fiberglass filters using a multiharvester and counted using standard liquid scintillation techniques. Results, expressed in cpm, are the average of quadruplicate cultures of cells pooled from four mice.

2.8. Cytokine measurement

Single cell suspensions from inguinal lymph nodes were resuspended at a final concentration of 5×10^6 cells/ml and cultured in 1 ml aliquots in 24-well tissue culture plates either in medium alone or with 50 μg/ml hen egg lysozyme. 48 h later, supernatants were harvested and stored at −70°C until assayed. Cytokine production was quantified using sandwich ELISA techniques. Briefly, supernatants were added to 96-well microtiter plates, previously coated overnight at 4°C with 100 μl of anti-interleukin-4 (4 μg/ml) and anti-interferon-γ(4 μg/ml) antibodies (Pharmingen) in 0.1 M $NaHCO_3$ buffer. Plates were then washed twice with PBS containing 0.05% Tween 20, after which non-specific protein-binding sites were blocked by incubation with 100 μl of PBS containing 1% ovalbumin at 37°C for 1 h. After blocking, the plates were washed three times and samples and standards (recombinant murine interleukin-4 and interferon-γ) (Pharmingen) were added to each well in a volume of 100 μl and incubated at 37°C for 1 h. Plates were washed three times and 100 μl/well biotinylated anti-murine interleukin-4 (2 μg/ml) and interferon-γ (2 μg/ml) antibodies (Pharmingen) diluted in PBS/1% ovalbumin was added. After incubation at 37°C for 1 h, the plates were washed three times and 100 μl/well streptavidin-alkaline phosphatase (Pharmingen) was added at 2 μg/ml. The plates were washed before 100 μl of *p*-nitrophenylphosphate was added to each well. Plates were read at 405 nm using automatic microplate reader. Cytokine levels were determined with reference to a standard curve constructed using serial dilutions of the standard cytokines and results are expressed in pg/ml.

3. Results

3.1. Treatment with an anti-interleukin-4 monoclonal antibody blocks the suppression of delayed type hypersensitivity responses to hen egg lysozyme in orally tolerized mice

Oral tolerance was induced in mice by feeding 0.1, 1 and 10 mg of hen egg lysozyme on days −5, −4, −3, −2 and −1 before immunization with the antigen on day 0. PBS and 10 mg of keyhole limpet hemocyanin were given as controls. The results showed that feeding either PBS or keyhole limpet hemocyanin induced marked delayed type hypersensitivity responses to hen egg lysozyme in footpads on day 12 (Fig. 1). In contrast, a reduction of the footpad delayed type hypersensitivity response was observed in mice fed hen egg lysozyme in a dose-dependent manner. The treatment with 1 mg of an anti-interleukin-4 monoclonal antibody at 30 min before each administration of hen egg lysozyme significantly blocked the suppression of the footpad swelling in mice fed either 1 or 10 mg of the antigen. The degree of the blockade by the monoclonal antibody of the suppression of the delayed type hypersensitivity response appeared to be greater in mice fed 10 mg of hen egg lysozyme than those given 1 mg of the antigen. There was no significant effect of the interleukin-4-neutralizing antibody on the footpad swelling in mice fed PBS, keyhole limpet hemocyanin, or 0.1 mg of hen egg lysozyme.

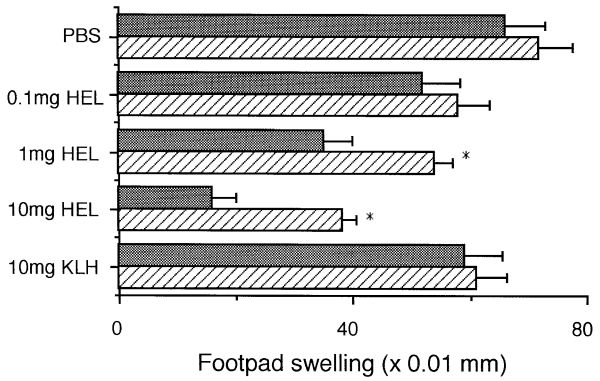

Fig. 1. Effect of an anti-interleukin-4 monoclonal antibody on the delayed type hypersensitivity response to hen egg lysozyme in mice fed varying doses of the antigen. To tolerize mice, the indicated doses of hen egg lysozyme (HEL) were orally administered on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. PBS and 10 mg of keyhole limpet hemocyanin (KLH) were given as feeding controls. To test the effect of an anti-interleukin-4 monoclonal antibody on the oral tolerance, PBS (stippled bar) and 1 mg of the monoclonal antibody (hatched bar) were i.p. injected 30 min before each oral administration of HEL. Footpad delayed type hypersensitivity responses to HEL were tested on day 12 as described in Section 2. Values are expressed as mean ± S.E.M. of six mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

The effect of varying doses of the monoclonal antibody against interleukin-4 on the suppression of the footpad delayed type hypersensitivity response was also examined in mice fed 10 mg of hen egg lysozyme. PBS and normal rat IgG were used as treatment controls. As shown in Fig. 2, mice treated with 0.01, 0.1 and 1 mg of the anti-interleukin-4 monoclonal antibody had diminished footpad delayed type hypersensitivity responses to hen egg lysozyme in a dose-dependent manner. The control rat IgG treatment failed to influence the suppression of the delayed type hypersensitivity.

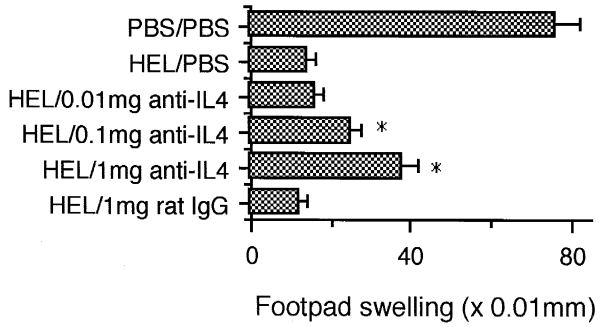

Fig. 2. Effect of varying doses of an anti-interleukin-4 monoclonal antibody on the delayed type hypersensitivity response to hen egg lysozyme in mice fed the antigen. To tolerize mice, 10 mg of hen egg lysozyme (HEL) was orally administered on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. PBS was given as a feeding control. To test the effect of an anti-interleukin-4 monoclonal antibody on the oral tolerance, 0.01, 0.1 and 1 mg of the monoclonal antibody were i.p. injected 30 min before each oral administration of HEL. PBS and 1 mg of normal rat IgG were given as treatment controls. Footpad delayed type hypersensitivity responses to HEL were tested on day 12 as described in Section 2. Values are expressed as mean ± S.E.M. of six mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

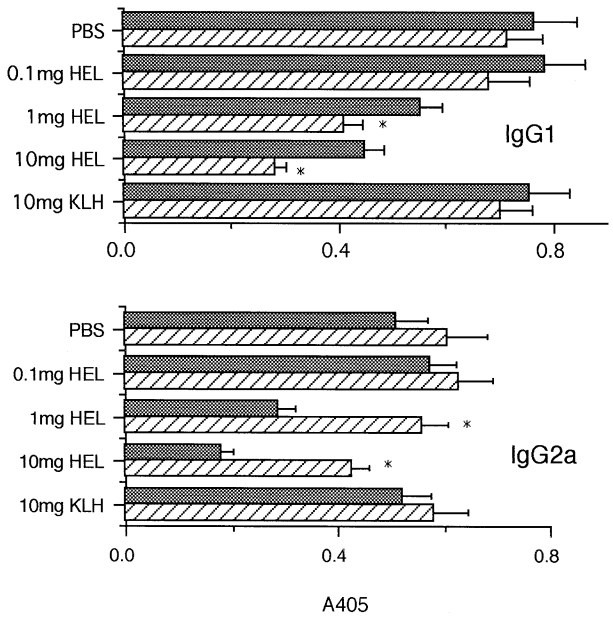

Fig. 3. Effect of an anti-interleukin-4 monoclonal antibody on the production of IgG1 and IgG2a antibodies to hen egg lysozyme in mice fed varying doses of the antigen. To tolerize mice, the indicated doses of hen egg lysozyme (HEL) were orally administered on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. PBS and 10 mg of keyhole limpet hemocyanin (KLH) were given as feeding controls. To test the effect of an anti-interleukin-4 monoclonal antibody on the oral tolerance, PBS (stippled bar) and 1 mg of the monoclonal antibody (hatched bar) were i.p. injected 30 min before each oral administration of HEL. Serum samples were collected on day 21 after immunization and individually assayed for anti-HEL IgG1 and IgG2a antibodies by ELISA as described in Section 2. Values are expressed as mean ± S.E.M. of five mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

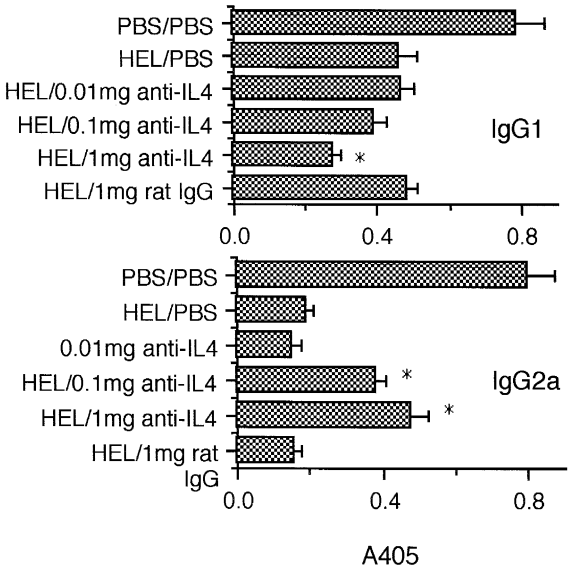

Fig. 4. Effect of varying doses of an anti-interleukin-4 monoclonal antibody on the production of IgG1 and IgG2a antibodies to hen egg lysozyme in mice fed the antigen. To tolerize mice, 10 mg of hen egg lysozyme (HEL) was orally administered on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. PBS was given as a feeding control. To test the effect of an anti-interleukin-4 monoclonal antibody on the oral tolerance, 0.01, 0.1 and 1 mg of the monoclonal antibody were i.p. injected 30 min before each oral administration of HEL. PBS and 1 mg of normal rat IgG were given as treatment controls. Serum samples were collected on day 21 after immunization and individually assayed for anti-HEL IgG1 and IgG2a antibodies by ELISA as described in Section 2. Values are expressed as mean ± S.E.M. of five mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

3.2. Treatment with an anti-interleukin-4 monoclonal antibody blocks the suppression of anti-hen egg lysozyme IgG2a antibody production, but facilitates the suppression of the IgG1 antibody production in orally tolerized mice

The production of IgG1 and IgG2a antibodies to hen egg lysozyme was significantly inhibited by the oral administration of the antigen, although the inhibition of the IgG2a antibody production appeared to be greater than that of the IgG1 antibody production (Fig. 3). Orally administered keyhole limpet hemocyanin showed no effect on the production of both isotypes of anti-hen egg lysozyme antibody. The injection of an anti-interleukin-4 monoclonal antibody further significantly lowered the serum levels of IgG1 antibody in mice fed either 1 or 10 mg of hen egg lysozyme. In contrast, marked increases in IgG2a antibody production were observed in mice treated with the monoclonal antibody.

Fig. 4 shows that 1 mg, but not other lower doses, of an anti-interleukin-4 monoclonal antibody significantly facilitated the suppression of anti-hen egg lysozyme IgG1 antibody production achieved by the oral administration of the antigen, while 0.1 and 1 mg of the monoclonal antibody markedly blocked the suppression of IgG2a isotype anti-

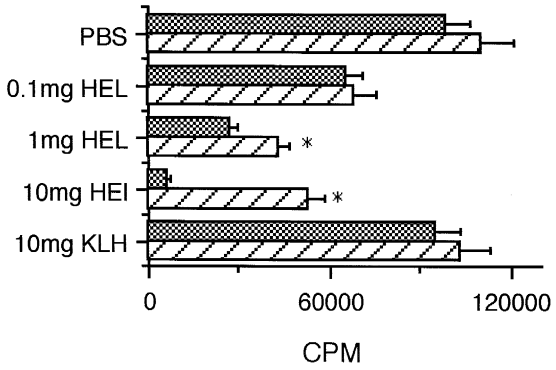

Fig. 5. Effect of an anti-interleukin-4 monoclonal antibody on the proliferative response to hen egg lysozyme in mice fed varying doses of the antigen. To tolerize mice, the indicated doses of hen egg lysozyme (HEL) were orally administered on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. PBS and 10 mg of keyhole limpet hemocyanin (KLH) were given as feeding controls. To test the effect of an anti-interleukin-4 monoclonal antibody on the oral tolerance, PBS (stippled bar) and 1 mg of the monoclonal antibody (hatched bar) were i.p. injected 30 min before each oral administration of HEL. Proliferative responses to HEL in mice were tested on day 14 as described in Section 2. Values are expressed as mean ± S.E.M. of quadruplicate cultures of cells pooled from 4 mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

body production. Neither anti-hen egg lysozyme IgG1 nor IgG2a antibody production in orally tolerized mice was affected by the treatment with normal rat IgG.

3.3. Treatment with an anti-interleukin-4 monoclonal antibody blocks the suppression of proliferative responses to hen egg lysozyme in orally tolerized mice

The proliferative response of lymph node cells to hen egg lysozyme was suppressed by the oral administration of

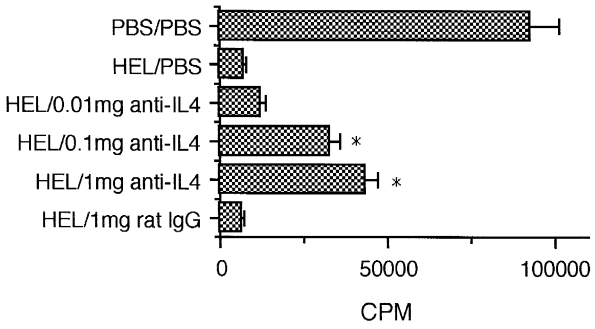

Fig. 6. Effect of varying doses of an anti-interleukin-4 monoclonal antibody on the proliferative response to hen egg lysozyme in mice fed the antigen. To tolerize mice, 10 mg of hen egg lysozyme (HEL) was orally administered on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. PBS was given as a feeding control. To test the effect of an anti-interleukin-4 monoclonal antibody on the oral tolerance, 0.01, 0.1 and 1 mg of the monoclonal antibody were i.p. injected 30 min before each oral administration of HEL. PBS and 1 mg of normal rat IgG were given as treatment controls. Proliferative responses to HEL in mice were tested on day 14 as described in Section 2. Values are expressed as mean ± S.E.M. of quadruplicate cultures of cells pooled from 4 mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

Table 1
Effect of an anti-interleukin-4 monoclonal antibody on the secretion of interleukin-4 and interferon-γ in mice tolerized orally

Group	IL-4 (pg/ml)	IFN-γ (pg/ml)
PBS/PBS	240 ± 18	8680 ± 582
HEL/PBS	183 ± 13	660 ± 42
HEL/0.01 mg anti-IL-4	149 ± 12	726 ± 55
HEL/0.1 mg anti-IL-4	82 ± 6 *	2192 ± 186 *
HEL/1 mg anti-IL-4	27 ± 1 *	4629 ± 140 *
HEL/1 mg rat IgG	176 ± 16	710 ± 68

The indicated doses of an anti-interleukin-4 (IL-4) monoclonal antibody were i.p. injected into mice fed 10 mg of hen egg lysoyme (HEL) on days −5, −4, −3, −2 and −1 before immunization with HEL on day 0. IL-4 and interferon (IFN)-γ secreted by cells of lymph nodes that were removed on day 14 were measured by sandwich ELISA as described in Section 2. Values are shown as mean ± S.E.M. of quadruplicate samples from culture supernatants of cells pooled from 4 mice. Anti-interleukin-4 monoclonal antibody treatment compared with PBS treatment (Student's *t*-test, * $P < 0.05$).

the antigen in a dose-related fashion, while the keyhole limpet hemocyanin feeding had no effect on the proliferative response (Fig. 5). When mice were treated with an anti-interleukin-4 monoclonal antibody, the suppression of the cell proliferation was markedly diminished. As shown in Fig. 6, a dose-related blockade of the suppression of the proliferative response to hen egg lysozyme in tolerant mice was achieved by the monoclonal antibody treatment.

3.4. Treatment with an anti-interleukin-4 monoclonal antibody blocks the suppression of interferon-γ secretion, but facilitates the suppression of interleukin-4 secretion by lymphoid cells from orally tolerized mice

The effect of an anti-interleukin-4 monoclonal antibody on the in vitro secretion of interleukin-4 and interferon-γ by lymphoid cells of orally tolerized mice was also investigated. Mice fed hen egg lysozyme had lower levels of secretion of both interleukin-4 and interferon-γ compared to those orally given PBS (Table 1). However, the suppression of the interferon-γ secretion was much greater than that of the interleukin-4 secretion (92% versus 24% of suppression rates). The treatment of the tolerant animals with an anti-interleukin-4 monoclonal antibody facilitated the suppression of interleukin-4 secretion in a dose-related fashion (up to 89%). In contrast, the suppression of the interferon-γ production was diminished dose-dependently by the treatment with the interleukin-4-neutralizing monoclonal antibody (down to 47%). The injection of control rat IgG into the tolerant mice had no effect on the levels of both cytokines.

4. Discussion

The present study demonstrates that the treatment of mice with an anti-interleukin-4 monoclonal antibody

blocked the suppression by oral hen egg lysozyme administration of the delayed type hypersensitivity response to the lysozyme, anti-hen egg lysozyme IgG2a antibody production, the antigen-specific proliferative response and interferon-γ secretion, while the suppression of anti-hen egg lysozyme IgG1 antibody production and interleukin-4 secretion was facilitated in the animals. To our knowledge, this is the first report of the modulation of oral tolerance by anti-interleukin-4 antibodies.

Th1 cells, a subset of $CD4^+$ T-cells, are crucial in the induction of delayed type hypersensitivity and IgG2a antibody production (Th1 responses) (Fong and Mosmann, 1989). Therefore, our finding that the delayed type hypersensitivity response to hen egg lysozyme and the antigen-specific IgG2a antibody production were suppressed by the oral antigen appears to support the previous result that Th1 cells are readily tolerized orally (Burstein et al., 1992). Furthermore, our data that the abrogation of the Th1 responses by an anti-interleukin-4 monoclonal antibody suggests that interleukin-4 plays a role in Th1 oral tolerance. Especially, it is of note that the induction of oral tolerance by feeding the high dose 10 mg of hen egg lysozyme was significantly blocked by the interleukin-4-neutralizing monoclonal antibody since other investigators reported that the induction of anergy was the mechanism of high dose oral tolerance (Melamed and Friedman, 1993; Friedman and Weiner, 1994), although they did not examine the effect of anti-interleukin-4 antibodies on the tolerance.

The secretion of the Th2 cytokine interleukin-4 itself was suppressed by the oral administration of hen egg lysozyme, but the suppression of the cytokine production was only partial (24% of a suppression rate), while the production of the Th1 cytokine interferon-γ was mostly suppressed by the oral antigen (92%). Therefore, the remaining amount of interleukin-4 in the tolerant mice might have contributed to the suppression of the delayed type hypersensitivity response to hen egg lysozyme and the antigen-specific IgG2a antibody production since interleukin-4 has been shown to suppress Th1 responses (Isakson et al., 1982; Peleman et al., 1989). Treatment with an anti-interleukin-4 monoclonal antibody facilitated the suppression of interleukin-4 production (up to 89%) that was associated with the diminution of the suppression of interferon-γ production (down to 47%). Therefore, the increased interferon-γ production as well as the decreased interleukin-4 production might have resulted in blocking the suppression of anti-hen egg lysozyme IgG2a antibody production and the delayed type hypersensitivity response to hen egg lysozyme since interferon-γ upregulates Th1 responses (Fong and Mosmann, 1989). Our results are supported by the previous finding that anti-interleukin-4 antibodies increase delayed type hypersensitivity responses as well as interferon-γ secretion, but decrease interleukin-4 secretion (Cheever et al., 1995; Jain et al., 1996), although the effect of the antibodies on the secretion of the both cytokines in orally tolerized animals has not been examined previously.

There was no significant effect of the treatment with an anti-interleukin-4 monoclonal antibody on the delayed type hypersensitivity response to hen egg lysozyme and the antigen-specific antibody production in mice orally given PBS, indicating that the monoclonal antibody did not influence immune responses to the antigen themselves. This is probably because the administration of the anti-interleukin-4 antibody was terminated one day before immunization with hen egg lysozyme and subsequently the antibody was cleared from the blood by the time of immunization.

Garside et al. showed that interleukin-4 deficient mice lacking IgG1 antibody production reduced antigen-specific IgG2a antibody production following the oral administration of a high dose antigen, as found in normal animals tolerized similarly, suggesting that interleukin-4 did not contribute to high dose oral tolerance (Garside et al., 1995). However, their results may not be directly comparable to those obtained by our studies since the considerable production of interleukin-4 was still seen in our tolerized mice with subsequent further reduction of the cytokine secretion following the interleukin-4 monoclonal antibody treatment, while interleukin-4 deficient mice genetically lack interleukin-4 production.

The results shown here support those obtained by Burstein and Abbas demonstrating the blockade by an anti-interleukin-4 antibody of the suppression of IgG2a and interferon-γ production in mice tolerized by i.p. injection of ovalbumin (Burstein and Abbas, 1993). However, in contrast with our finding that the interleukin-4-neutralizing antibody blocked the suppression of proliferative responses in orally tolerized mice, they failed to diminish the suppression of the lymphoid cell proliferation in the i.p tolerized animals. Therefore, they concluded that the reduced T-cell proliferation might be due to the direct T-cell anergy, not due to the interleukin-4 function. Thus, interleukin-4 may play a role in tolerance induced via the oral route, but not via the parenteral route.

The facilitated decrease in the Th2 cell-controlled anti-hen egg lysozyme IgG1 antibody production in mice treated with an anti-interleukin-4 monoclonal antibody appears to be due to the facilitated suppression by the monoclonal antibody of the secretion of interleukin-4 that plays a critical role in Th2 cell responses and to the upregulation by the anti-interleukin-4 antibody of the production of interferon-γ that suppresses Th2-mediated immune responses (Snapper and Paul, 1987; Finkelman et al., 1988).

A number of studies showed that feeding antigens readily induces tolerization of Th1 cells, while Th2 cells appeared to be resistant to oral tolerance (Melamed and Friedman, 1993; Fishman-Lobell et al., 1994). In contrast with these findings, recently Garside et al. demonstrated that a single high dose of antigens reduced, to equal degrees, the production of antigen specific antibodies of

both the IgG2a and IgG1 isotypes (Garside et al., 1995). It was also recently shown that continuous feeding of antigens resulted in marked suppression of both Th1 and Th2 cells responses, while a single or intermittent feeding regimen was effective in inducing tolerance in Th1 cells, but not Th2 cells (Melamed et al., 1996). On the other hand, as shown in the present experiments, daily feeding low and high doses of hen egg lysozyme for 5 consecutive days induced marked tolerization of Th1 cells and moderate tolerization of Th2 cells. The discrepancies among these findings may reflect differences in the strains of mice used, the nature of the antigens, or the frequency of antigen administration. More work appears to be required before concluding the precise mechanism of oral tolerance.

In summary, treatment with an anti-interleukin-4 monoclonal antibody seems to be effective in blocking the suppression of Th1 responses including the delayed type hypersensitivity response to hen egg lysozyme, the antigen-specific IgG2a antibody production and interferon-γ secretion in mice fed either low or high doses of the antigen, while the suppression of Th2 responses including anti-hen egg lysozyme IgG1 antibody production and interleukin-4 secretion was facilitated in the animals. These results further suggest that interleukin-4, at least in part, plays a role in low and high dose Th1 oral tolerance.

References

Burstein, H.J., Abbas, A.K., 1993. In vivo role of interleukin 4 in T-cell tolerance induced by aqueous protein antigen. J. Exp. Med. 177, 457–463.

Burstein, H.J., Shea, C.M., Abbas, A.K., 1992. Aqueous antigens induce in vivo tolerance selectively in IL-2- and IFN-γ-producing (Th1) cells. J. Immunol. 148, 3687–3691.

Cheever, A.W., Finkelman, F.D., Cox, T.M., 1995. Anti-interleukin-4 treatment diminishes secretion oTh2 cytokines and inhibits hepatic fibrosis in murine schistosomiasis japonica. Parasite Immunol. 17, 103–109.

Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., Weiner, H.L., 1994. Regulatory T-cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. Science 265, 1237–1240.

Chen, Y.H., Inobe, J., Marks, R., Gonnella, P., Kuchroo, V.K., Weiner, H.L., 1995. Peripheral deletion of antigen-reactive T-cells in oral tolerance. Nature 376, 177–180.

Enavall, E., Perlmann, P., 1972. Enzyme-linked immunosorbent assay. III. Quantitation of specific antibodies of enzyme labeled anti-immunoglobulin in antigen-coated tubes. J. Immunol. 109, 129–135.

Finkelman, F.D., Katona, M., Mosmann, R.R., Coffman, R.L., 1988. IFN-gamma regulates the isotypes of Ig secreted during in vivo humoral immune responses. J. Immunol. 140, 1022–1027.

Fishman-Lobell, J., Friedman, A., Weiner, H.L., 1994. Different kinetic patterns of cytokine gene expression in in vivo in orally tolerant mice. Eur. J. Immunol. 24, 2720–2724.

Fong, T.A., Mosmann, T.R., 1989. The role of IFN-gamma in delayed type hypersensitivity mediated by Th1 clones. J. Immunol. 143, 2887–2893.

Friedman, A., Weiner, H.L., 1994. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc. Natl. Acad. Sci. USA 91, 6688–6692.

Garside, P., Steel, M., Worthey, E.A., Satoskar, A., Alexander, J., Bluethmann, H., Liew, F.Y., Mowat, A.M., 1995. T helper 2 cells are subject to high dose oral tolerance and are not essential for its induction. J. Immunol. 154, 5649–5655.

Higgins, P., Weiner, H.L., 1988. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. J. Immunol. 140, 440–445.

Howard, M., Farrar, J., Hilfiker, M., Johnson, B., Takatu, K., Hamaoka, T., Paul, W.E., 1982. Identification of a T-cell derived B-cell growth factor distinct from interleukin-2. J. Exp. Med. 155, 914–921.

Isakson, P.C., Pure, E., Vitetta, S., Krammer, P.H., 1982. T-cell-derived B-cell differentiation factor(s): Effect on the isotype switch of murine B-cells. J. Exp. Med. 155, 734–748.

Jain, S.L., Barone, K.S., Michael, J.G., 1996. Activation patterns of murine T-cells after oral administration of an enterocoated soluble antigen. J. Exp. Med. 167, 170–175.

Khoury, S., Hancock, W.W., Weiner, H.L., 1992. Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of tranforming growth factor B, interleukin 4 and prostaglandin E expression in the brain. J. Exp. Med. 176, 1335–1364.

Lider, O., Santos, L.M.B., Lee, C.S.Y., Higgins, P.J., Weiner, H.L., 1989. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and in vitro immune responses is mediated by antigen-specific CD8+ T-lymphocytes. J. Immunol. 142, 748–752.

Melamed, D., Friedman, A., 1993. Direct evidence for anergy in T-lymphocytes tolerized by oral administration of ovalbumin. Eur. J. Immunol. 23, 935–942.

Melamed, D., Fishman-Lobell, J., Uni, Z., Weiner, H.L., Friedman, A., 1996. Peripheral tolerance of Th2 lymphocytes induced by continuous feeding of ovalbumin. Int. Immunol. 8, 717–724.

Mosmann, T.R., Coffman, R.L., 1989. Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. 7, 145–173.

Mowat, A.M., 1987. The regulation of immune responses to dietary protein antigens. Immunol. Today 8, 93–95.

Mowat, A.Mcl., 1993. Oral tolerance and regulation of immunity to dietary antigens. In: Ogra, P.L., Mestecky, J., Lann, M.E., Strober, W., McGhee, J.R., Bienesstock, J. (Eds.), Handbook of Mucosal Immunology. Academic Press, San Diego, CA, pp. 185–210.

Nagler-Anderson, C., Bober, L.A., Robinson, M.E., Siskind, G.W., Thorbecke, F.J., 1986. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. Proc. Natl. Acad. Sci. USA 83, 7443–7446.

Nussenblatt, R.B., Caspi, R.R., Mahdi, R., Chan, C., Roberge, F., Lider, O., Weiner, H.L., 1990. Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral induction of tolerance with S-antigen. J. Immunol. 144, 1689–1695.

Ohara, J., Paul, W.E., 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. Nature 315, 333–336.

Peleman, R., Wu, J., Fargeas, C., Delespersse, G., 1989. Recombinant interleukin-4 suppresses the production of interferon-γ by human mononuclear cells. J. Exp. Med. 170, 1751–1756.

Snapper, C.M., Paul, W.E., 1987. Interferon-gamma and B-cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science 236, 944–947.

Weiner, H.L., Friedman, A., Miller, A., Khoury, S.J., Al-Sabbagh, A., Santos, L., Sayegh, M., Nussenblatt, R.B., Trentham, D.E., Hafler, D.A., 1994. Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu. Rev. Immunol. 12, 809–837.

Whitacre, C.C., Gienapp, I.E., Orosz, C.G., Bitar, D., 1991. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. J. Immunol. 147, 2155–2163.