Journal of Korean Medical Science
Vol. 11, No. 2, 111-117 April 1996

Anti-IL-4 Antibody Inhibits Antigen Specific IgE Response but Fails to Prevent Chicken Gamma Globulin-induced Active Systemic Anaphylaxis : Evidence for the Involvement of IgG antibodies

Hern-Ku Lee, Hwang-Ho Lee, Young-Min Park, Hyung-Ju Park, Jeong-Ho Lee, and Tai-You Ha

Department of Microbiology and Immunology, Chonbuk National University Medical School, Chonju, Chonbuk 560-182, Republic of Korea

It has recently been reported that interleukin-4 (IL-4) is required for the production of IgE, and anti-IL-4 monoclonal antibody (mAb) inhibits in vivo IgE responses. These suggest that blocking of IL-4 activity may be useful for the prevention or treatment of immediate hypersensitivity disorders. In this study we investigated whether anti-IL-4 has a regulatory role in chicken-gamma globulin (CGG)-induced active systemic anaphylaxis, Multiple injections of anti-IL-4 (up to 40 mg / mouse) failed to protect the mice from fatal anaphylaxis. Anti-IL-4 strongly suppressed CGG-specific IgE response (> 90 %) without any suppressive effect on CGG-specific IgG(IgG1, IgG2a, IgG2b, and IgG3) responses. Because these data suggest the possibility that fatal anaphylaxis could be induced by IgG antibodies, we examined the possibility using anti-CGG polyclonal and the subclasses of IgG monoclonal antibodies. Passive sensitization of mice with polyclonal antibodies elicited severe and fatal anaphylactic shock; about 50 % of the mice died. The activity of antibodies was not diminished by heat treatment (56°C, 2h), suggesting that the anaphylaxis was not mediated by IgE. Shock was also elicited by each subclass of IgG mAb; of these, IgG1 was the most effective. Combination of the IgG subclasses elicited more exaggerated shock; about 30 % of mice died. These data indicate that IgG antibodies are themselves sufficient to induce systemic anaphylaxis. Therefore, the failure of anti-IL-4 to prevent active anaphylaxis is probably due to the inability of anti-IL-4 to suppress the production of IgG antibodies.

Key Words : *Anti-IL-4, IL-4, Chicken gamma globulin, IgE, IgG, Anaphylaxis*

INTRODUCTION

IgE antibodies play a central role in immediate hypersensitivity (Ishizaka and Ishizaka, 1967 ; Ishizaka and Ishizaka, 1975). Suppression of IgE production has long been considered to be the ideal treatment

Address for correspondence : *Tai-You Ha, Department of Microbiology and Immunology, Chonbuk National University Medical School, Chonju, 560-182, Chonbuk.*
Tel : (0652)70-3067, Fax : (0652)74-9866.

for patients with allergy. The approaches which have been studied include the use of immunological reagents (Finkelman et al., 1988 ; Marshall and Bell, 1989 ; Souillet et al., 1989), immunosuppressive drugs (Okudaria et al., 1986), and modified allergens (Katz et al., 1973 ; Takasu and Ishizaka, 1975 ; Lee and Sehon, 1977 ; Hayglass and Stefura, 1991), but the practical implications of such approaches remain unclear.

Recently, interleukin-4 (IL-4) has been shown to stimulate murine as well as human IgE synthesis both in vitro and in vivo (Finkelman et al., 1988 ; Pene et al., 1988 ; Snapper et al., 1988 ; Dekruyff et al., 1989 ; Vercelli et al., 1989). Furthermore, anti-IL-4 monoclonal antibody (mAb) has been shown to block the primary and secondary in vivo IgE responses (Finkelman et al., 1986 ; Finkelman et al., 1988 ; Finkelman et al., 1989 ; Finkelman et al., 1990 ; Sadick et al., 1990 ; Ochel et al., 1991). This suggests that regulation of IL-4 production or action may be useful for the prevention or therapy of immediate hypersensitivity disorders.

The purpose of this study was to investigate whether in vivo administration of anti-IL-4 could prevent active systemic anaphylaxis induced by chicken-gamma globulin(CGG).

MATERIALS AND METHODS

Animals

Female BALB/c mice were purchased from the Korean Institute for Chemistry (Taejon, Chungnam). Female athymic nude BALB/c mice were kindly provided by Dr. Huh, Korean Cheil Sugar R & D Center (Dokpyong, Kyonggi), and housed in the specific pathogen-free animal care facility in our laboratory. All mice were used at 8-12 wks of age.

Antibodies

The rat-mouse hybridoma, 11B11, which secretes rat IgG1 specific for murine IL-4 (Ohara and Paul, 1985) was purchased from American Type Culture Collection (Rockville, MD). As a control mAb, J4-1(Finkelman et al., 1988) which secretes rat IgG1 with specificity for the hapten nitrophenol (NP), was kindly provided by Dr. F.D. Finkelman, Uniformed Services University of the Health Sciences (Bethesda, MD). Both mAbs were prepared as ascites in pristane-primed nude mice. Preparations were precipitated in 45 % ammonium sulfate, dialyzed against PBS, pH 7.2, and quantitated for protein before use (Lowry et al., 1951).

Anti-CGG polyclonal antibodies were produced by a method described by Tung et al(1978). Briefly, BALB/c mice were immunized 3 times on day 0, 14, and 21 by i.p. injections of 0.2 ml mixture of 9 parts of Freund's complete adjuvent(CFA) and 1 part of CGG solution (50 mg/ml). The developed ascites were collected and a gamma-globulin rich fraction was prepared with ammonium sulfate and protein concentration was measured as described above.

CGG-specific mouse IgG1, IgG2a, IgG2b, IgG3 and IgE mAbs were produced. A 6 weeks old BALB/c mouse was immunized by s.c injection of 50 μg of CGG with CFA (1 : 1, v/v). Two weeks later, another 50 μg of CGG was injected i.v. Three days later, spleen cells were prepared and fused with NS-1 mouse myeloma cells with polyethylene glycol 4,000 as described previously (Shulman et al., 1978 ; Mckearn et al., 1979). Hybrids were selected with HAT medium. Screening of mAb-producing hybrids was performed by an ELISA in which microtiter plates were coated with CGG (20 μg/ml) and their subclasses were determined using mAb isotyping kit. The positive clones were selected and cloned 3 times by limiting dilution. The mAbs were prepared in BALB/c mice. The concentrations of IgG subclasses in ascitic fluid were measured by a radial immunodiffusion assay with standard mouse IgG and rabbit anti-mouse IgG (Cappel Laboratories, Malvern, PA). IgE concentrations in ascitic fluid were measured by a sandwich ELISA (Xia et al., 1989) using goat anti-mouse IgE (Bethyl Laboratories, INC. Montgomery, TX), mouse IgE mAb specific for dinitrophenol (ICN ImmunoBiologicals, Lisle, IL), and peroxidase-conjugated rat anti-mouse IgE (Biosource, Westlarke Village, CA).

Determination of CGG-specific serum Ig levels

CGG-specific serum IgG1, IgG2a, IgG2b, IgG3, and IgE levels were determined using an ELISA calibrated against anti-CGG mAbs. ELISA plates were coated overnight with CGG at 20 μg/ml in carbonate buffer (0.05 M, pH 9.6). After the plate were washed, serial dilutions of serum samples were incubated for 1 hr for IgG or 4 hrs for IgE at 37℃, the plates were washed, and appropriately diluted peroxidase-conjugated rabbit anti-mouse IgG subclasses

(Cappel Laboratories) or rat anti-mouse IgE were added and incubated for 2 hrs at 37℃. After washing the plates extensively, substrate solution was added. After optimal color development, the reaction was stopped with 4 N H_2SO_4. Absorbance at 492 nm was measured in an ELISA reader. Results were expressed as concentration from a standard curve of known concentrations of CGG-specific mAbs.

Reagents

A mouse mAb isotyping kit was purchased from The Binding Site Inc. (San Diego, CA). CGG and OVA(Grade V) were from Cappel Laboratories and Sigma (St. Louis, MO), respectively. CFA was from GIBCO (Grand Island, nNY). Recombinant human IL-2 and murine IL-4 were gifts from Hoffman-La Roche, Inc (Nutley, NJ), and Dr. Paul Trotta, Schering Corp. (Bloomfield, NJ), respectively.

Induction of Active systemic anaphylaxis

BALB/c mice were sensitized by i.p. injection of 500 μg of CGG, 1.0 mg of alum and 2 × 10^9 *Bordetella pertussis* (National Institute of Health, Korea) as described previously (Ha and Reed, 1986; Ha and Reed, 1987). Challenge was performed by i.v. injection of 500 μg CGG 18 days after sensitization. Thirty minutes after challenge, shock was scored by McCaskill's criteria (McCaskill et al., 1984) with some modifications : 0, no sign of shock; 1, decreasing bouts of spontaneous activity and piloerection; 2, loss of coordination and dyspnea; 3, no activity following whisker stimuli and slight activity after prodding with ball-point pen; 4, no activity following whisker stimuli, progressive paresis beginning with the hind leg; 5, no activity following Haffner's tail pinching stimuli by forcep; 6, brief but violent convulsion, prostration, coma but substantial recovery; 7, fatal shock (died within 15-60 min); 8, fatal shock (died within 15 min).

Induction of passive systemic anaphylaxis

BALB/c mice were injected i.v. with 1 mg of polyclonal or 2 mg of monoclonal IgG antibodies. The mice thus sensitized for 1 hr and then tested for anaphylactic shock by i.v. challenge with 500 μg of CGG. Shock was scored as described above.

RESULTS

Failure of anti-IL-4 to prevent ASA induction

An activity of anti-IL-4 was determined using murine CTLL cell proliferation assay (Lee et al., 1990). A 50 % inhibition of CTLL proliferation in response to 20 U of murine IL-4 was observed at concentration of the mAb as low as 10 ng/ml, whereas high concentration (up to 200 μg/ml) of anti-IL-4 did not affect IL-2-induced proliferation (data not shown).

To investigate whether anti-IL-4 is able to prevent systemic anaphylaxis, mice were sensitized with CGG and 2.5 mg, 5 mg or 10 mg of anti-IL-4 or 10 mg of anti-NP were injected i.p. 4 times at 3 day intervals from the day of sensitization (saline for control group). Results from three separate experiments are summurized in Table 1. Seventy-four percent of control mice died of shock. However, there was no significant difference in severity of anaphylaxis between control and anti-IL-4- or anti-NP-treated groups. Anti-IL-4, even at a high concentration (a total of 40 mg), did not protect the mice from the fatal anaphylaxis.

Table 1. Anti-IL-4 has no protective activity on the CGG-induced active systemic anaphylaxis

mAb[a]	Concentration(mg)	No. of mice	No. of mice showing the following shock score[b]								
			0	1	2	3	4	5	6	7	8
—	—	24	0	0	0	0	2	3	2	8	9
Anti-IL-4	10	19	0	0	1	0	2	3	0	5	8
Anti-Il-4	20	20	0	0	0	0	2	2	1	7	8
Anti-IL-4	40	21	0	0	1	2	2	3	0	7	6
Anti-NP	40	7	0	0	0	0	1	1	0	2	3

[a] : mAbs were injected i.p. 4 times, at 3 day intervals, from the day of sensitization. The amount of mAbs shown in Table is the total amount injected.

[b] : Scoring criteria are described in Materials and Methods.

Table 2. Anti-IL-4 blocks in vivo CGG-specific serum IgE but not IgG responses

Group[a]	mAb[b]	CGG-specific Ig(μg/ml)[c]				
		IgE	IgG1	IgG2a	IgG2b	IgG3
I	—	6.8±2.1	186.0±16.2	28.0±3.2	17.1±2.0	11.1±0.8
II	Anti-IL-4	0.6±0.1	201.8±28.3	51.0±5.5	33.6±4.9	26.7±3.2
III	Anti-NP	5.2±1.3	183.2±21.7	22.4±1.8	21.7±1.8	16.6±2.0

[a] : Mice (six to eight/group) were sensitized with 500 μg of CGG plus 1.0 mg of alum and 2 $\times 10^9$ *Bordetella pertussis*.

[b] : Mice were injected i.p. with either saline or 10 mg of anti-IL-4 or anti-NP for 4 times at 3 day intervals from the day of sensitization.

[c] : Partial bleeding was conducted on day 12, and CGG-specific IgG levels were determined by ELISA, and shown in μg/ml. Data are shown as mean±S.D.

Anti-IL-4 suppressed CGG-specific IgE but not IgG responses.

CGG-specific serum IgE as well as IgG levels were determined on day 12, the time of peak response in our experimental conditions. The data in Table 2, the mean of data from three independent experiments, demonstrated that serum IgE levels were suppressed by 90 % in anti-IL-4 treated mice (40 mg), but were not altered in the anti-NP-treated group (40 mg), as compared with saline-treated group. By contrast to the significant suppression of the IgE response by anti-IL-4, the IgG response was not inhibited at all and there were increases in the IgG2a, IgG2b, and IgG3 responses (ranging from 82 % to 140 %) by the antibody. Anti-NP had no significant effect on IgG responses.

Induction of passive systemic anaphylaxis by polyclonal and monoclonal IgG antibodies

The data described above suggested two possibilities ; i) since anti-IL-4 did not completely suppressed IgE response, a low level of IgE is sufficient to induce fatal anaphylaxis, or ii) Ig other than IgE may be participated in the induction of the reaction. To prove the latter possibility, we prepared anti-CGG polyclonal antibodies and all subclasses of IgG mAbs and tested whether these antibodies were able to induce passive anaphylaxis. As shown in Fig. 1, all mice which had been sensitized passively with polyclonal antibody displayed severe signs of anaphylatic shock greater than grade 5 ; about 50 % of the mice died of shock. The antibody was then heated for 2 hrs at 56℃ to remove IgE activity (MaCamish, 1967). This treatment of the antibody did not diminish its activity, suggesting that the antibody class responsible for the anaphylaxis may not be IgE but IgG. To prove this, mice were sensitized with each subclass of anti-CGG

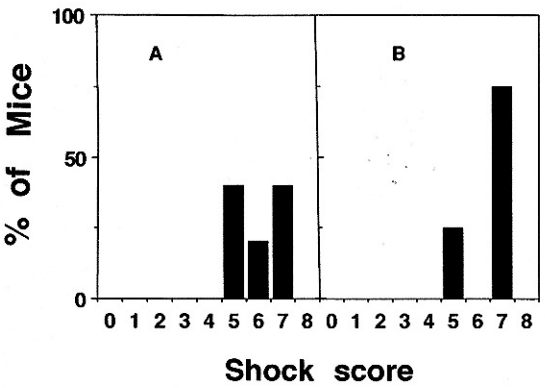

Fig. 1. Induction of passive systemic anaphylaxis by anti-CGG polyclonal antibodies. BALB/c mice (ten/group) were injected i.v. with 1 mg of either untreated (A) or heat-treated (B) (56℃, 2h) anti-CGG polyclonal antibodies. One hour later, the mice were challenged with 500 μg of CGG.

IgG mAb. As shown in Fig. 2, the majority of mice which were sensitized with IgG1 displayed moderate signs of shock corresponding to grade 3, 4 and 5, whereas mice sensitized with other IgG subclasses showed mild shock signs (less than grade 3). Next, the effect of combinations of all IgG subclasses was examined. As shown in Fig. 3, combinations of all IgG subclasses elicited more exaggerated shock signs than those by each subclass ; about 30 % of mice died and the remaining showed shock signs greater than grade 4. When IgG1 was excluded from the combination, the reaction was no more than that of each IgG2a, IgG2b or IgG3. The reactions elicited by both anti-CGG polyclonal and monoclonal antibodies were antigen specific, because no reaction was elicited when mice were challenged with heterologous antigen, i.e., OVA (data not shown). These data clearly indicate that IgG antibodies are sufficient to

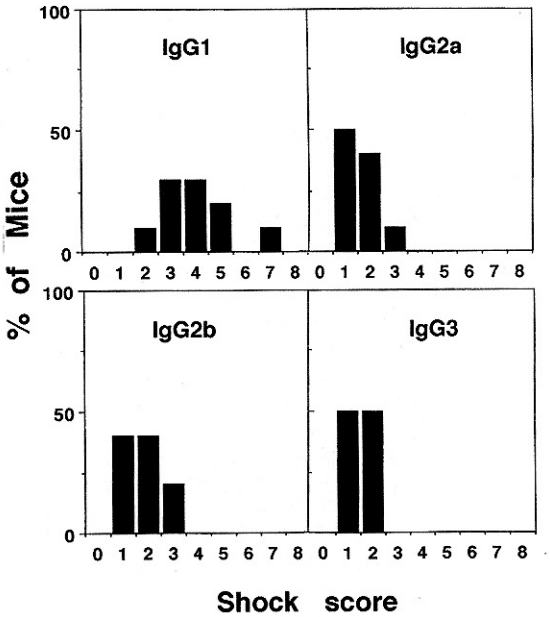

Fig. 2. Induction of passive systemic anaphylaxis by anti-CGG IgG mAbs. BALB/c mice (ten/group) were injected i.v. with 2 mg of each subclass of IgG mAb. One hour later, the mice were challenged with 500 μg of CGG.

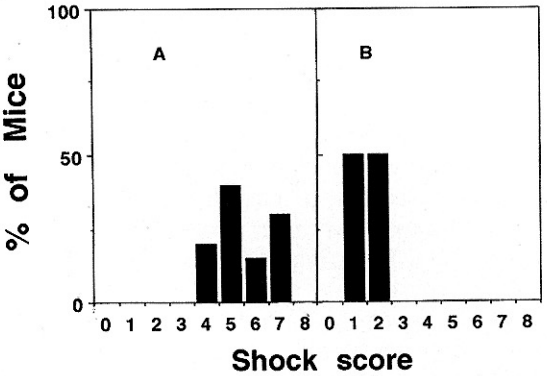

Fig. 3. Loss of severe anaphylactic reactions by exclusion of IgG1 mAbs. BALB/c mice (ten/group) were injected i.v. with 2 mg of combinations of all subclasses of IgG mAb (0.5 mg of each subclass) (A) or 2 mg of combinations of three subclasses (IgG2a + IgG2b + IgG3) of IgG mAbs (B). One hour later, the mice were challenged with 500 μg of CGG.

induce lethal anaphylaxis and IgG1 is the most potent in inducing the anaphylaxis.

DISCUSSION

Our results show that in vivo administration of a large quantity of anti-IL-4 (40 mg/mouse) fails to protect mice from CGG-induced active systemic anaphylaxis. This concentration of anti-IL-4 significantly suppressed anti-CGG IgE but not IgG responses. Fatal passive systemic anaphylaxis was elicited by CGG-specific polyclonal antibodies and heat treatment (56℃, 2 h) of the antibodies did not reduce their anaphylaxis-inducing activities, suggesting that the class of antibody responsible for the anaphylaxis may not be IgE. Therefore, we tested whether IgG antibodies can elicit systemic anaphylaxis using all subclasses of IgG mAb specific for CGG. A moderate shock by IgG1 and a mild shock by IgG2a, IgG2b or IgG3 were elicited. Injection of all subclasses of IgG resulted in induction of a more exaggerated shock; about 30 % of the mice died. These results clearly indicate that a fatal anaphylaxis is easily elicited by IgG antibodies. Therefore, the failure of anti-IL-4 to prevent ASA is probably due to its inability to suppress IgG responses.

The finding that anti-IL-4 suppressed IgE but not IgG responses is in agreement with those reported by Finkelman et al., (Finkelman et al., 1986; Finkelman et al., 1988; Finkelman et al., 1989; Finkelman et al., 1990) who showed that in vivo administration of anti-IL-4 inhibited IgE without effect on IgG1 or IgG2a responses in mice infected with *Nippostrongylus brasiliensis* or injected with goat anti-mouse IgD, but differs from those obtained in another in vivo studies in which anti-IL-4 significantly reduced the increase in serum IgG1 levels (Schurmans et al., 1990; Ochel et al., 1991). These differences might be due to the use of different experimental systems.

In fact, the involvement of IgG antibodies in systemic anaphylaxis in mice has also been reported by other investigators. Munoz and Bergman (1977) found that fatal anaphylaxis was transferred with purified IgG1, but not IgE. Recently, Arimura et al.(1990) reported that passive systemic anaphylaxis was elicited by IgG1 mAb in mast cell-deficient mice. However, nobody has yet shown which subclass of IgG is participated or effective in the induction of systemic anaphylaxis as we carried out in this study.

It is generally accepted that IgE is the antibody class responsible for anaphylatic reaction. However,

our findings that i) there was no differences in severity of shock in spite of significant suppression of IgE responses (Table 1 and 2), ii) the shock-inducing capacity of polyclonal antibodies was not diminished by IgE inactivation (Fig. 1) and iii) more importantly, our unpublished data showing that neither of the two different monoclonal IgE preparations with specificity for CGG was able to elicit shock signs in inbred BALB/c, C57BL/6, and ICR mice (1 mg of IgE/mouse) tempted us to speculate that the antibody class responsible for systemic anaphylaxis in this experimental system may not be IgE but IgG. Therefore, it is resonable to speculate that the occurrence of fatal anaphylaxis in anti-IL-4-treated mice is due to IgG antibodies which are not suppressed by anti-IL-4.

In conclusion, our study showed that in vivo administration of anti-IL-4 failed to prevent CGG-induced active systemic anaphylaxis in BALB/c mice, and the failure seems to be attributable to the inability of anti-IL-4 to suppress IgG antibodies which can induce active systemic anaphylaxis, indicating that simply blocking IL-4 activity is not sufficient to prevent anaphylaxis in which IgG antibodies are participated.

ACKNOWLEDGEMENT

This work was supported by the Korean Science and Engineering Foundation grant 890701-20. We thank Soon-OK Roh for her expert technical assistance and for typing the manuscript.

REFERENCES

Arimura A, Nagato M, Watanabe A, Nakamura K, Takeuchi M, Jarada M. *Production of active and passive anaphylactic shock in the WBB6F1 mouse, a mast cell-deficient strain. Experinentia 1990; 46: 739-42.*

DeKruyff RH, Turner T, Abrams JS, Palladino MA Jr, Umetsu DT. *Induction of human IgE synthesis by CD4$^+$ T cell clones: requirement for interleukin 4 and low molecular weight B cell growth factor. J Exp Med 1989; 170: 1477-93.*

Finkelman FD, Holmes J, Katona IM, Uraban JF Jr, Beckmann MP, Park LS, Schooley KA, Coffman RL, Mossmann TR, Paul WE. *Lymphokine control of in vivo imunoglobulin isotype selection. Annu Rev Immunol 1990; 8: 303-33.*

Finkelman FD, Holmes J, Uraban JF Jr, Paul WE, Katona IM. *T help requirement for the generation of an in vivo IgE response: a late acting form of T cell help other than IL-4 is required for IgE but not for IgG1 production. J Immunol 1988; 142: 403-8.*

Finkelman FD, Katona IM, Uraban JF, Holmes J, Ohara J, Tung AS, Sample JG, Paul We. *IL-4 is required to generate and sustain in vivo IgE responses. J Immunol 1989; 141: 2335-41.*

Finkelman FD, Katona IM, Uraban JF, Snapper CM Jr, Ohara J, Paul WE. *Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. Proc Natl Acad Sci USA 1986; 83: 9675-8.*

Ha TY, Reed ND. *Immune response potential of mast cell-deficient W/W^v mice. Int Arch Allergy appl Immunol 1986; 80: 85-94.*

Ha TY, Reed ND. *Systemic anaphylaxis in mast cell-deficient mice of W/W^v and S1/S1^d genotypes. Expl Cell Biol 1987; 55: 63-8.*

Hayglass KT, Stefura W. *Antigen-specific inhibition of on going murine IgE responses II: Ihibition of IgE responses induced by treatment with glutaraldehyde-modified allergens is paralleled by reciprocal increases in IgG2a synthesis. J Immunol 1991; 147: 2455-60.*

Ishizaka K, Ishizaka T. *Identification of rE antibodies as a carrier of reagenic activity. J Immunol 1967; 99: 1187-98.*

Ishizaka T, Ishizaka K. *Biology of immunoglobulin E: molecular basis of reagenic hypersensitivity. Prog Allergy 1975; 19: 60-121.*

Katz DH, Hamaoka T, Benacerraf B. *Induction of immunological tolerance in bone marrow-derived lymphocytes of the IgE antibody class. Proc Natl Acad Sci USA 1973; 70: 2776-80.*

Lee HK, Xia X, Choi YS. *IL-4 blocks the up-regulation of IL-2 receptors induced by IL-2 in normal human B cells. J Immunol 1990; 144: 3431-6.*

Lee WY, Sehon AH. *Abrogation of reagenic antibodies with modified allergens. Nature 1977; 267: 618-9.*

Lowry PH, Rosebrough NJ, Farr AL, Randall R J. *Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-9.*

MaCamish J. *A heat labile-skin sensitizing activity of mouse serum. Nature 1967; 214: 1228-9.*

Marshall JS, Bell E. *Induction of an auto-anti-IgE response in rats III. Inhibition of a specific IgE response. Immunology 1989; 66: 428-33.*

McCaskill AC, Hosking DS, Hill DJ. *Anaphylaxis following intranasal challenge of mice sensitized with ovalbumin. Immunology. 1984; 51: 669-77.*

McKeam TJ, Fitch FW, Smilek DE, Sarmiento M, Stuart FB. *Properties of rat anti-MHC antibodies produced by cloned rat-mous hybridomas. Immunol Rev 1979; 47: 91-115.*

Munoz J, Bergman R. *Bordetella pertusis: Immunological and other biological activities. Immunological Series, Vol 4, New York: Marcel Dekker Inc, 1977.*

Ochel M, Vohr HW, Pfeiffer C, Gleichmann E. *IL-4 is required for the IgE and IgG1 increase and IgG1 autoantibody formation in mice treated with mercuric*

chloride. J Immunol 1991; 146: 3006-11.

Ohara J, Paul WE. *Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. Nature 1985; 315: 333-6.*

Okudaira H, Sakurai Y, Terada K, Tearda E, Ogita T, Miyamoto T. *Cyclosporon A-induced suppression of ongoing IgE antibody formation. Int Arch Allergy appl Immunol 1986; 79: 164-8.*

Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, Yokota T, Arai N, Arai KI, Banchereau J, De Vries E. *IgE production by normal human lymphocytes is induced by interleukin-4 and suppressed by interferons γ and α and prostaglandin E2. Proc Natl Acad Sci USA 1988; 85: 6880-4.*

Sadick MD, Heinzel FP, Holaday BJ, Pu RT, Dawkins RS, Locksley RM. *Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody: evidence for a T cell-dependent, interferon-γ-independent mechanism. J Exp Med 1990; 171: 115-27.*

Schurmans S, Heusser CH, Qin HY, Merino J, Brighouse G, Lambert PH. *In vivo effects of anti-IL-4 monoclonal antibody on neonatal induction of tolerance and on an associated autoimmune syndrome. J Immunol 1990; 145: 2465-73.*

Shulman M, Wilde CD, Kohler G. *A better cell line for making hybridomas secreting specific antibodies. Nature. 1978; 276: 269-70.*

Snapper CM, Finkelman FD, Paul WE. *Differential regulation of IgG1 and IgE synthesis by interleukin-4. J Exp Med 1988; 167: 183-96.*

Souillet G, Rousset F, De Vries JE. *Alpha interferon treatment of patients with hyper IgE syndrome. Lancet 1989; i: 1384.*

Takatsu K, Ishizaka K. *Reagenic antibody formation in mouse VI. Suppression of IgE and IgG antibody responses to ovalbumin following the administration of high dose urea-denatured antigen. Cell Immunol 1975; 20: 276-89.*

Tung AS, Chiorazzi N, Katz DH. *Regulation of IgE antibody production by serum molecules. I. Serum from complete Freund adjuvant-immune donors suppresses irradiation-enhanced IgE production in low responder mouse strains. J Immunol 1978; 120: 2050-9.*

Vercelli D, Jabara HH, Arai KI, Geha. *Induction of human IgE synthesis requires IL-4 and T/B interactions involving the T cell receptor/CD3 complex and MHC class II antigens. J Exp Med 1989; 169: 1295-307.*

Xia X, Lee Hk, Clark SC, Choi YS. *Recombinant interleukin (IL)-2-induced human B cell differentiation is mediated by autocrine IL-6. Eur J Immunol 1989; 19: 2275-81.*