

IMMUNOGENIC PROPERTIES OF MODIFIED ANTIGEN E

II. Ability of Urea-Denatured Antigen and α -Polypeptide Chain to Prime T Cells Specific For Antigen E¹

KIMISHIGE ISHIZAKA, HIROKAZU OKUDAIRA, AND TE PIAO KING

From the Department of Medicine, The Johns Hopkins University School of Medicine at the Good Samaritan Hospital, Baltimore Md., 21239 and The Rockefeller University, New York, N.Y. 10021

Ragweed antigen E loses its major antigenic determinant after denaturation in 8 M urea. The urea-denatured (UD) antigen and α -polypeptide chain isolated from the denatured molecule possess their own antigenic determinant(s) but lack the major determinant of the native molecule. The UD antigen and α -chain, however, are capable of priming mouse T cells specific for antigen E. Priming of A/J mice with the modified antigen enhanced both IgG and IgE antibody responses to antigen E. Both UD antigen-primed spleen cells and α -chain-primed spleen cells collaborate with DNP-primed cells to give an adoptive secondary anti-DNP antibody response to DNP-ragweed antigen in syngeneic irradiated mice. Pretreatment of A/J mice with an i.v. injection of α -chain partially suppressed both IgE and IgG antibody responses to antigen E. Weekly injections of α -chain or UD antigen to antigen E-primed animals depressed on-going IgE antibody response, and suppressed secondary IgE antibody response to antigen E. Transfer of spleen cells from animals treated with the modified antigen to irradiated recipients followed by challenge with native antigen showed that the adoptive secondary response was suppressed by injections of modified antigen to the donors. The results indicate that the immunocompetent cell population was changed by the treatment and provide an experimental model to analyze the immunologic effect of hypsensitization treatment.

Previous work by King *et al.* (1) have shown that the major antigenic determinants of ragweed antigen E are lost by denaturation in 8 M urea. The urea-denatured (UD)² antigen as well as the two polypeptide chains, α and β , isolated from the denatured molecules failed to induce allergic skin reactions in ragweed-sensitive individuals (1) and, did not combine with rabbit and human antibodies specific for antigen E (2). In view of the accumulated evidence which indicates that precursors of antibody-forming cells have antigen receptors with the same specificity as the antibodies they produce (3, 4), one can expect that UD antigen, as well as polypeptide chains, would not react with the major population of B cells specific for antigen E. Our

previous findings, however, showed that these modified antigens stimulate T cells specific for the native antigen (2). It was found that mesenteric lymph node cells primed with dinitrophenylated ragweed Fraction (Fr) D (DNP-Rag) produced an anti-DNP antibody response upon stimulation with DNP-keyhole limpet hemocyanin (KLH) followed by culture in the presence of either native antigen E or one of the modified antigens such as UD antigen, α -chain and β -chain. It appears that the modified antigen stimulated carrier (Rag)-specific helper cells to release enhancing factor and triggered DNP-specific cells which had combined with DNP-KLH. It was also found that the modified antigens were capable of stimulating lymphocytes of ragweed-sensitive patients for DNA synthesis, although the patients did not have antibody against the modified antigen (α -chain). The results indicated that the modified antigens possess immunogenic determinants capable of reacting with antigen E-primed T cells.

The present experiments were undertaken to study whether the UD antigen and/or α -chain are capable of priming antigen E-specific T cells of mice. The results will show that these modified antigens have immunogenicity and prime T cells specific for antigen E. Subsequent experiments will show that injections of the modified antigens into antigen E-primed animals depressed on-going IgE antibody formation and a secondary IgE antibody response to native antigen E.

MATERIALS AND METHODS

Antigens. Fr D of Rag was prepared by the method of King *et al.* (5) from low ragweed pollen (Greer Laboratories, Inc., Lenoir, N. C.). Antigen E preparation employed for immunization and radioimmunoassay was obtained from the National Institutes of Health, Bethesda, Md. The urea-denatured antigen and α -chain of antigen E were the same preparations as those employed in a previous study (2). Antigen E was isolated by the ammonium sulfate gradient solubilization procedures (6) and was used to prepare the modified antigens. As shown in a previous article (2), possible contamination of the modified antigens with native antigen E was less than 0.1%. KLH was purified from the blood of limpets (Pacific Bio-Marine Supply Co., Venice, Calif.) by the method of Campbell *et al.* (7). Crystalline human serum albumin (HSA) was purchased from Nutritional Biochemical Corp., Cleveland, Ohio. Dinitrophenyl derivatives of ragweed Fr D, KLH, and HSA were prepared as described in a previous article (8). The number of DNP groups per milligram of DNP-Rag and DNP-KLH was 2.3×10^{-7} mole and 7×10^{-7} mole, respectively. The DNP-HSA contained 20 DNP groups per molecule.

Antisera. Rabbits were immunized by repeated injections of antigen E included in aluminum hydroxide gel (9) or complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.).

Submitted for publication June 25, 1974.

¹This work was supported by research Grants GB-41443 from the National Science Foundation, AI-10060, and AI-08445 from The U. S. Public Health Service. This is publication #138 from the O'Neill Laboratories at The Good Samaritan Hospital.

²Abbreviations used in this paper: UD, urea-denatured; KLH, Keyhole limpet hemocyanin; HSA, human serum albumin; Fr, Fraction; DNP-Rag, dinitrophenylated Fr D of ragweed pollen extract; CFA, complete Freund's adjuvant; PCA, passive cutaneous anaphylaxis; RGG, rabbit γ -globulin.

All antisera gave a single precipitin band of identical specificity with Fr D and antigen E by gel diffusion precipitin analysis. Some antisera obtained by the immunization with CFA gave a faint precipitin band against α -chain, whereas those obtained by the immunization with alum failed to do so. An antiserum from a CFA-immunized rabbit was employed as a standard antiserum for the measurement of anti-antigen E and anti- α -chain antibodies.

A rabbit antiserum against mouse γ -globulin was the same preparation as that employed in a previous article (10). A goat antiserum specific for rabbit IgG (RGG) was obtained by immunization with the Fc fragment of RGG included in CFA.

Immunization and adoptive transfer. Female A/J strain mice of 20 to 25 g (Jackson Laboratories, Bar Harbor, Maine) were employed. Unless specified, mice were immunized by an i.p. injection of an appropriate antigen mixed with aluminum hydroxide gel, which was prepared by the method of Roveltella and Ovary (9). They were bled from the retroorbital plexus and serum antibody levels were determined as described below.

Adoptive transfer experiments were carried out as described in a previous article (11). Donors of carrier-specific cells were immunized with 10 μ g of antigen E, UD antigen or α -chain in saline, or 1 μ g of the antigen included in 1 mg alum. Donors of DNP-primed cells were immunized with 1 μ g DNP-KLH mixed with 1 mg alum 3 weeks before the transfer. Spleen cells from a given type of primed donor mice were suspended in Hanks' balanced salt solution, washed twice and transferred i.v. into four syngeneic recipients, which had been irradiated 24 hr before the cell transfer. X-rays were administered at a dose of 600 R with a Ridge 300 KVp biologic x-ray irradiator (Tucker, Georgia). Usually 10^7 cells of a given type were administered to each recipient. Secondary antigen challenge was carried out by an i.p. injection of either 10 μ g DNP-Rag or 10 μ g antigen E to the recipients immediately after the cell transfer. Mice were bled 7, 10, 14, 21, and 28 days after the immunization and pooled serum from four mice were titrated for antibodies.

Radiolabeling of antigens. Antigen E and α -chain were labeled with ^{125} I by the method of Klinman and Taylor (12) with slight modifications. Specifically, 2 to 2.5 mCi of ^{125} I (carrier-free high specific activity, Cambridge Nuclear Inc., Billerica, Mass.) was used to label 30 to 50 μ g of each antigen. The antigen in a volume of 20 μ l was mixed with 50 μ l of 0.2 M borate buffer, pH 8.6, and 10 μ l of ^{125} I solution. Ten microliters of chloramine-T (10 mg/ml) in the borate buffer were then added to the tube, kept for 60 sec at room temperature and the oxidizing reagent in the mixture was reduced by addition of an excess amount of sodium metabisulfite. After 1 min, 1 mg of HSA in acetate buffer, pH 6.0, was added to reduce the pH to neutral. DNP-HSA was labeled with ^{125} I by the same method, except that 1 mCi of ^{125} I was used to label 500 μ g of the antigen. As reported by Yagi *et al.* (13), antigen E was effectively labeled at alkaline pH. After extensive dialysis against borate-buffered saline, pH 8.0, a proportion of total radioactivity associated with antigen E was determined with a rabbit antiserum against antigen E. One-tenth milliliter of a 1:100 dilution of the radio-labeled antigen E in 0.1% HSA, containing approximately 80 ng/ml of protein, was mixed with an equal volume of 5 μ g/ml of cold antigen E. One-tenth milliliter of the antigen solution (2.5 μ g/ml) was mixed with an equal volume of a serial 2-fold dilutions (1:50 to 1:3200 dilution) of the antiserum in 20 μ gN/ml of normal rabbit IgG (RGG). After incubation at 4°C overnight, an excess amount of goat anti-RGG was added to precipitate RGG. Radioactivity of the precipitate formed from antibody excess mixtures showed that

65% of radioactivity in the ^{125} I-antigen E preparation was associated with antigen E. As radioactivity of the precipitate was proportional to the concentration of anti-serum in the range of 1:800 to 1:3200 dilution, the antigen binding activity of the antiserum was calculated, assuming that 65% of total radioactivity in the antigen preparation corresponded to the concentration (2.5 μ g/ml) of antigen E. The antigen-binding activity of the antiserum was estimated to be 825 μ g/ml. Since the rabbit antiserum contained antibody against α -chain, radioactivity associated with α -chain in the ^{125} I-labeled α -chain preparation was determined by a similar manner. The results showed that 55% of total radioactivity was associated with α -chain and that the α -chain-binding activity of the antiserum was 38.4 μ g/ml. More than 95% of radioactivity in the ^{125} I-DNP HSA preparation was bound to anti-DNP antibody.

Measurement of antibodies. The antigen-binding activity of IgG antibody was determined by double antibody technique. Detailed procedure of the test and a standard serum for anti-DNP antibody were described in a previous article (9). The test was set up in duplicate. For the measurement of anti-DNP antibody, 0.1 ml of a 1:100 dilution of serum samples were incubated with an equal volume of 2 μ gN/ml of ^{125} I-DNP HSA, antigen-antibody complexes were coprecipitated with γ -globulin by adding an excess amount of anti-mouse γ -globulin. Radioactivity of the precipitates was measured by automatic gamma counter (Nuclear Chicago Inc., Des Plaines, Ill.) The antigen-binding activity of the serum samples was determined by interpolating the radioactivity of the precipitate into a standard curve.

The measurement of anti-antigen E antibody and anti- α -chain antibody were carried out by the same method, except that the concentration of ^{125} I-labeled antigen used for the test was in the order of 10 ng/ml. For each test, a standard curve was set up with a rabbit antiserum (see above) and goat antiserum specific for RGG. The radioactivity of mouse γ -globulin-anti-mouse IgG precipitate was interpolated into the standard curve and the antigen-binding activity of undiluted mouse serum was calculated, in comparison with the activity of the rabbit antiserum to bind the same antigen.

The IgE antibody was titrated by passive cutaneous anaphylaxis (PCA) reactions in the rat (Holtzman Co., Madison, Wis.) (14). PCA reactivity given by anti-DNP and by anti-antigen E antibodies were elicited with a 2-hr sensitization period by an i.v. injection of 4 mg DNP-HSA or 2 mg of ragweed Fr D in 1 ml of 1% Evans blue. The PCA reaction with anti- α -chain antibody was induced by local challenge of sensitized skin sites with 0.1 μ g/ml of α -chain, followed by an i.v. injection of Evans blue. Comparisons between the i.v. challenge and local challenge were made with the antigen E system to confirm that local challenge with 0.1 μ g/ml of antigen E and an i.v. challenge with 2 mg Fr D gave comparable PCA titer.

The PCA reaction was set up in duplicate or triplicate for each sample and the titer was expressed by a geometric mean of serum dilutions required for giving a positive skin blueing. In both i.v. challenge and local challenge methods, variation of the titer for the same sample was less than 2-fold.

RESULTS

The immunogenicity of UD antigen and α -chain was studied. A group of four mice was immunized with 1 μ g of antigen E, UD antigen, or α -chain included in 1 mg alum. They were bled at 1-week intervals and both IgG and IgE antibodies against native antigen or α -chain in their sera were measured.

The results of IgG antibody response are shown in Table I. In the animals immunized with antigen E, IgG antibody against the homologous antigen became detectable at 2 weeks and their antigen-binding activity increased to 21 $\mu\text{g}/\text{ml}$ at 4 weeks. The IgE antibody against antigen E reached a maximum (1:160) at 2 weeks and the titer persisted for 6 weeks. Neither IgG nor IgE antibody against α -chain was detectable in the serum. Animals immunized with either α -chain or UD antigen failed to form a detectable amount of IgG and IgE antibody against either α -chain or antigen E by 4 weeks. After a booster immunization with the same dose of antigen as that used for primary immunization, these mice formed IgG antibodies but no detectable IgE antibody against α -chain and antigen E. It appears that the modified antigens are immunogenic but their immunogenicity is low as compared with that of the native antigen. An immunization with 10 μg UD antigen or α -chain included in CFA resulted in the formation of much IgG antibody against α -chain and a small amount of the antibody against antigen E. As shown in Table I, the concentration of anti-antigen E antibody in their serum was only 2 to 5% of the anti- α -chain antibody. The results indicated that most of the antibody formed by the immunization with either UD antigen or α -chain failed to combine with the native antigen, indicating that anti- α -chain and anti-antigen E antibodies have different specificities. IgE antibody against α -chain was detected in serum 2 to 3 weeks after a booster injection with the same antigen in CFA but the PCA titer did not exceed 1:40.

Experiments were undertaken to study whether the UD antigen and/or α -chain can prime T cells specific for antigen E. The first experiment was carried out to see the effect of priming of mice with a modified antigen on the antibody response to native antigen. A group of four mice was primed by an i.p. injection of 10 μg UD antigen or α -chain without adjuvant. After 2 weeks, these animals as well as unprimed animals were immunized with 1 μg of antigen E included in 1 mg alum. Animals were bled 7, 10, 14, 21, and 28 days after the immunization and both IgG and IgE antibodies against antigen E were determined. The results of the experiments are shown in Figure 1. In the animals primed with either one of the modified antigens, IgE antibody appeared in the serum at day 7 when the antibody was undetectable in control mice. The kinetics of antibody response was more rapid in the modified antigen-primed animals, although the magnitude of the antibody response was not significantly greater than that in the unprimed control. The results also showed a definite enhancement of IgG antibody response in the primed animals. These

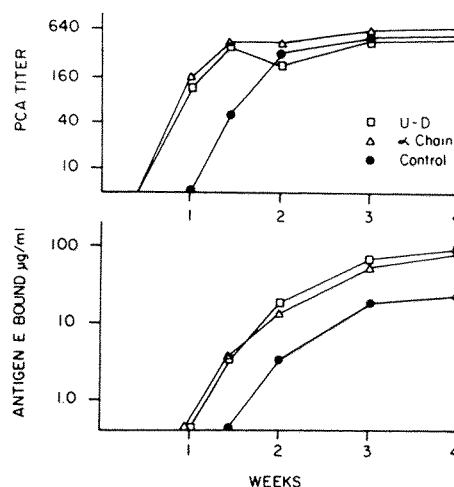


Figure 1. Effect of priming with UD antigen or α chain on the antibody response to antigen E. Groups of 4 mice received an intraperitoneal injection of either UD antigen or α chain. Two weeks later, these mice and normal control mice were immunized with 1 μg antigen E included in 1 mg alum.

findings are similar to "carrier effect" which was observed in the immunization with hapten-carrier conjugate (10, 15). Experiments were designed to show the specificity of T cell priming. A group of mice was primed with either UD antigen or α -chain. At day 14, these animals and control mice were immunized with 1 μg DNP-KLH included in alum. In all of the three groups, anti-DNP IgE and IgG antibodies became detectable at day 10 and 14, respectively. The antibody titer at 2 and 3 weeks were comparable in the three groups.

Priming of T cells by modified antigen was confirmed by adoptive transfer experiments. Donors of helper cells were primed with 1 μg of antigen E, UD antigen, or α -chain included in 1 mg alum. A group of animals was immunized with 0.01 μg antigen E in 1 mg alum. Two weeks after the immunization, these animals were sacrificed. Ten million spleen cells from each group of donors or normal spleen cells were mixed with 2×10^6 spleen cells from DNP-KLH-primed animals and the cells were transferred i.v. into irradiated syngeneic recipients. They were challenged by an i.p. injection of 10 μg DNP-Rag. The results of the adoptive secondary anti-DNP responses of IgE and IgG classes are shown in Figure 2. The control group which received DNP-primed cells together with normal spleen cells failed to form a detectable amount of anti-DNP antibody, whereas the animals receiving either α -chain-primed cells or UD antigen-primed cells together with the hapten-primed cells showed both IgE and IgG antibody responses. It was also found that the helper function of α -chain-primed cells and UD antigen-primed cells was higher than that of spleen cells of donors which were primed with 0.01 μg antigen E. As a possible contamination of modified antigens with native antigen E was less than 0.1%, priming of T cells with modified antigen can not be explained by possible contamination of the antigen with native antigen E.

Priming of T helper cells with modified antigen suggested the possibility that the same antigens may induce T cell tolerance. In order to study this possibility, we injected 100 μg of α -chain, UD antigen, or antigen E i.v. into A/J mice. Twenty four hours later, these animals and control animals received 1 μg antigen E absorbed on 1 mg alum, and the antibody responses of both IgE and IgG classes were observed. Figure 3 shows that administration of antigen E or α -chain partially

TABLE I
Immunogenicity of urea-denatured antigen and α chain

Immunizing Antigen		Adjuvant	Antigen-Binding Activity ($\mu\text{g/ml}$)			
			4 Weeks		3 Weeks after booster	
			Anti- α	Anti-AgE	Anti- α	Anti-AgE
(μg)						
AgE	1.0	alum	<0.1	21.0	— ^a	—
UD ^b	1.0	alum	<0.1	<0.1	0.87	<0.1
α Chain	1.0	alum	<0.1	<0.1	4.2	0.3
UD	10.0	CFA	5.0	0.5	100	2.8
α Chain	10.0	CFA	103.0	1.3	102	2.5

^a Animals did not receive a booster injection.

^b UD represents urea-denatured antigen.

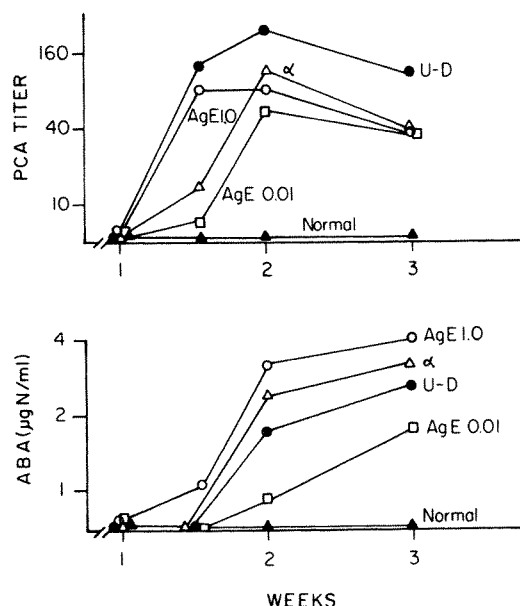


Figure 2. Adoptive anti-DNP antibody responses of IgE and IgG classes in irradiated mice. All groups received DNP-KLH-primed spleen cells. Four groups of donors of carrier-specific cells were immunized with 1.0 μ g or 0.01 μ g antigen E, 1.0 μ g UD antigen or 1.0 μ g α chain included in 1 mg alum. Control mice received normal spleen cells and DNP-KLH primed spleen cells. Recipients were challenged by 10 μ g DNP-Rag.

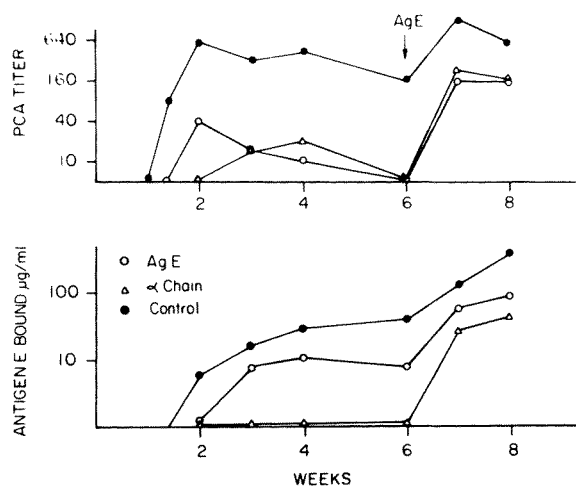


Figure 3. Suppression of antibody response to antigen E by an intravenous injection of antigen E or α chain. Twenty-four hr prior to immunization with 1 μ g antigen E included in 1 mg alum, two groups of mice received an intravenous injection of 100 μ g α chain or antigen E. These animals and control mice, which were immunized with 1 μ g antigen in alum, were boosted with 10 μ g antigen E in saline.

suppressed IgE antibody response to the native antigen. The treatment with α -chain suppressed IgG antibody response almost completely. An injection of UD antigen before immunization slightly suppressed IgE and IgG antibody responses, however, the suppression was less as compared with α -chain administration (not shown). Six weeks after the primary immunization with antigen E, all animals received 10 μ g antigen E in saline. As shown in Figure 3, all groups gave a secondary response to the booster injection, indicating that priming with antigen E was not suppressed by the previous administration of α -chain.

The possibility was considered that injections of modified antigens might affect the on-going antibody response to

antigen E. Three groups of mice were immunized with 1 μ g antigen E included in alum. At 2 weeks when the IgE antibody titer reached to maximum, two groups of animals received an i.v. injection of 10 μ g of either α -chain or UD antigen without adjuvant. The same modified antigen was injected i.p. once every week for 7 weeks. In these groups, the IgE antibody titer to antigen E began to decline after four injections and showed some discrepancy from the control animals which did not receive either antigen (Fig. 4). One week after the seventh injection of the modified antigen, both the treated and control animals were boosted with 10 μ g antigen E in saline. As shown in Figure 4, secondary IgE antibody response to the native antigen was suppressed in the two groups which had received weekly injections of the modified antigen. The treatment, however, did not significantly change the on-going IgG antibody response. In the animals treated with α -chain, IgG antibody titer was slightly higher than that of control animals. In addition to the antibody against antigen E, the animals treated with the modified antigen formed anti- α -chain antibody of IgG class. The antibody appeared after injections of either UD antigen or α chain. At the time of a booster injection, however, the concentration of the antibody did not reach to the level of IgG anti-antigen E antibody. Serum samples of the treated animals were tested for the presence of anti- α -chain antibody of IgE class. The antibody was not detectable either before or after the booster injection of antigen E.

Since the animals receiving the weekly injections of modified antigen had slightly higher titer of IgG antibody than control animals, an adoptive transfer experiment was carried out to eliminate a possible role of circulating antibody in the suppression of secondary IgE antibody response. Three groups of mice were immunized with 1 μ g antigen E in alum and two groups were treated by weekly i.p. injections of either UD antigen or α -chain. The immunization regimen was exactly the same as that described above, except that 20 μ g rather than 10 μ g of modified antigen was injected into each animal. Similar to the results of the previous experiment, IgE antibody titer began to decline after four injections of either α -chain or UD antigen. At 9 weeks after priming with antigen E, when the IgE anti-

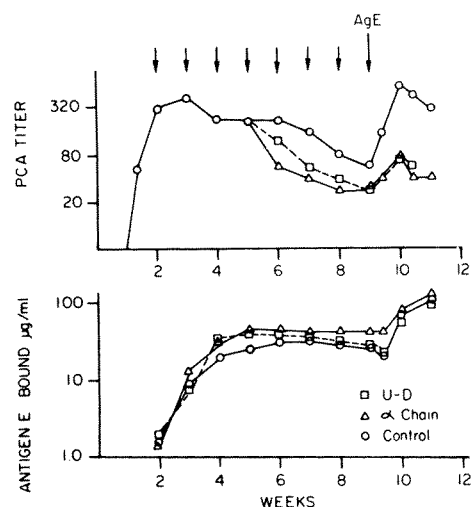


Figure 4. Effect of weekly injections of α chain or UD antigen on on-going antibody response to antigen E. All animals were immunized with 1 μ g antigen E plus 1 mg alum, and boosted with 10 μ g antigen E in saline at week 9. Either 10 μ g UD antigen or α chain were injected once a week beginning from week 2 to week 8. Control mice received saline.

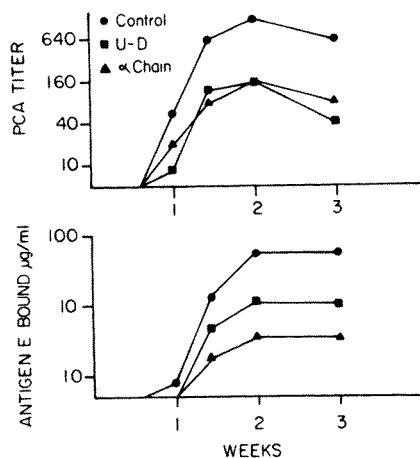


Figure 5. Adoptive secondary antibody response to antigen E. Animals primed with antigen E and treated in a similar manner as that shown in Fig. 4 were sacrificed at week 9. 10^7 spleen cells from each group were transferred into irradiated recipients, which were challenged with $10 \mu\text{g}$ antigen E. Spleen cells from untreated mice showed highest IgE and IgG antibody responses.

body titer in the treated animals were one-fourth to one-eighth of the control animals, they were sacrificed and 10^7 spleen cells from each group were transferred into irradiated animals. Recipients were challenged by an i.p. injection of $10 \mu\text{g}$ antigen E in saline and their antibody response was followed. Figure 5 shows that recipients of the cells from treated donors gave much less response of anti-antigen E by both IgE and IgG classes as compared with the recipients of spleen cells from control animals.

DISCUSSION

Data described in this paper clearly showed that both UD antigen and α -chain were immunogenic in mice but that their immunogenicity was poor as compared with that of native antigen E. No detectable IgG or IgE anti- α -chain antibody was formed by immunization with $1 \mu\text{g}$ modified antigen in alum. A booster injection of the same antigen in alum or a single injection of $10 \mu\text{g}$ of modified antigen in CFA resulted in the formation of antibodies against α -chain. The mice immunized with the modified antigen formed a small amount of antibody against antigen E, however, the antigen E-binding activity in the sera was less than 10% of their α -chain-binding activity, indicating that at least the major portion of anti- α -chain does not react with native antigen E. Absence of anti- α -chain antibody in the anti-antigen E mouse serum strongly suggests that mouse anti-antigen E fails to react with α -chain. A weak antigen E-binding activity in anti-UD serum and anti- α -chain serum may be due either to a contamination of the modified antigen preparations with a small amount (less than 0.1%) of native protein or to the presence of denatured protein in ^{125}I -labeled antigen E preparation. In any event, the results collectively indicate that a major population of B cells stimulated by native antigen is different from the majority of B cells stimulated by the modified antigen(s).

In spite of the different specificity between native antigen E and modified antigens, evidence was obtained that both native and modified antigens stimulated common T cells. Enhancement or acceleration of IgG and IgE antibody responses to antigen E by previous priming of the mice with either UD antigen or α -chain is comparable to "carrier effect" in anti-hapten antibody response. Furthermore, the results of the

adoptive transfer experiment showed that spleen cells of donors primed with a subimmunogenic dose (c.f. Table I) of UD antigen or α -chain collaborated with DNP-primed cells in irradiated recipients upon challenge with DNP-Rag. The fact that $1 \mu\text{g}$ of α -chain or UD antigen was more effective than $0.01 \mu\text{g}$ antigen E for the proliferation of 'carrier-specific' helper cells excluded the possibility that the effect of modified antigen-priming is due to contamination of the preparations with native antigen. The possibility may be considered that DNP-Rag might contain DNP coupled with denatured antigen E and that such a DNP-denatured antigen conjugate might be responsible for collaboration between α -chain-specific T cells and DNP-specific B cells. This possibility cannot be excluded, but is unlikely because of the following reasons. a) A 1-mg/ml of DNP-Rag preparation did not show a precipitin band with anti- α -chain whose α -chain-binding activity was more than $100 \mu\text{g}/\text{ml}$; b) immunization of mice with DNP-Rag in CFA resulted in the formation of both anti-DNP and anti-antigen E but not a detectable amount of anti- α chain. Our more recent experiments have shown that three monthly injections of DNP-Rag ($10 \mu\text{g}$) in 10 mg alum into the rabbit induced formation of both anti-DNP and anti-antigen E but no anti- α -chain.

The present experiment also showed that an i.v. injection of α -chain 24 hr before immunization with antigen E suppressed anti-antigen E antibody formation. The suppressive effect of α -chain was comparable to or higher than antigen E itself. In view of the fact that α -chain does not have major antigenic determinants in the antigen E molecules, mechanisms of the partial unresponsiveness could be similar to T cell tolerance which was described by Chiller *et al.* (16). The relatively weak suppressive effect of UD antigen is probably due to its molecular size. Antigen E is denatured and polymerized by urea denaturation (1). The UD antigen used in the present experiment was polymerized material separated from monomolecular antigen E by gel filtration. It has been shown that unresponsiveness to human γ -globulin or bovine γ -globulin by a previous administration of the antigen is difficult to obtain with aggregated material (17).

The most interesting observation in the present experiment was that on-going and persistent IgE antibody response to antigen E declined following weekly injections of either α -chain or UD antigen, and that the treatment suppressed secondary IgE antibody response to the native antigen (Fig. 4). The IgG antibody response, however, was not significantly affected by the treatment. Indeed, the IgG antibody concentration in the α -chain-treated animals was almost twice as high as the control animals which received no treatment, and secondary IgG antibody response in the treated animals was comparable to that observed in the untreated control. Suppression of the secondary IgE antibody response was not due to high IgG antibody level. It was found in adoptive transfer experiments that recipients of spleen cells from the treated animals formed less IgE and IgG antibody than the recipients of the cells from control animals (Fig. 5). It is apparent that weekly injections of the modified antigens changed the population of primed cells so that secondary response is depressed. Questions remain: 1) why IgG secondary response was not depressed in the *in vivo* experiment, whereas suppression was clearly demonstrated in the adoptive transfer experiment; 2) whether the depression is due to some changes in the T cell population or in the B cell population. Analysis of the primed cell population in the treated and untreated animals is under study. More recent

experiments, which will be reported separately, have shown that helper function of antigen E-primed spleen cells for the adoptive secondary anti-hapten antibody response of DNP-primed cells is diminished by weekly injections of the modified antigen. The results suggested that the antigen E-specific T cell population has been changed by the treatment.

In any event, depression of IgE antibody response to antigen E by weekly injections of modified antigen will provide an experimental model for hyposensitization treatment in hay fever patients. Our previous studies on ragweed-sensitive patients have shown that IgE antibody titer in more than one-half of the patients declined after a long term treatment, and almost all the treated patients failed to show secondary IgE antibody response after the ragweed season, whereas almost all untreated patients studied at the same time showed the secondary IgE antibody response (18). Since the decline of the IgE antibody titer did not correlate with IgG antibody in the serum of treated patients, we have speculated that the depression of the IgE antibody formation is not due to an increase of IgG antibody formation. The present experiments in the mouse indicate that some changes in the primed cell population can be induced by repeated stimulation of antigen-primed T cells and suggest that such a mechanism is involved in the immunologic effect of hyposensitization treatment.

Acknowledgment. The authors express their appreciation to Drs. Takashi Makinodan and Albert A. Nordin, National Institutes of Health, for irradiation of mice.

REFERENCES

1. King, T. P., Norman, P. S., and Tao, N., *Immunochemistry*, 11: 83, 1974.
2. Ishizaka, K., Kishimoto, T., Delespesse, G., and King, T. P., *J. Immunol.*, In press.
3. Wigzell, H. and Mäkelä, O., *J. Exp. Med.*, 132: 110, 1970.
4. Walters, C. S. and Wigzell, H., *J. Exp. Med.*, 132: 1233, 1970.
5. King, T. P. and Norman, P. S., *Biochemistry*, 1: 709, 1962.
6. King, T. P., *Biochemistry*, 11: 367, 1972.
7. Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdorf, D. H., *Methods in Immunology*, p. 69, W. A. Benjamin, New York, 1963.
8. Kishimoto, T. and Ishizaka, K., *J. Immunol.*, 111: 720, 1973.
9. Roveltella, R. and Ovary, Z., *Int. Arch. Allergy Appl. Immunol.*, 36: 282, 1969.
10. Ishizaka, K. and Okudaira, K., *J. Immunol.*, 110: 1067, 1973.
11. Okudaira, H. and Ishizaka, K., *J. Immunol.*, In press.
12. Klinman, N. R. and Taylor, R. B., *Clin. Exp. Immunol.*, 39: 156, 1969.
13. Yagi, Y., Maier, P., Pressman, D., Arbesman, C., and Reisman, R. E., *J. Immunol.*, 91: 33, 1963.
14. Mota, I. and Wong, D., *Life Science*, 8: 813, 1969.
15. Katz, D. H. and Benacerraf, B., *Adv. Immunol.*, 15: 1, 1972.
16. Chiller, J. M., Habicht, G. S., and Weigle, W. O., *Science*, 171: 813, 1971.
17. Weigle, W. O., *Adv. Immunol.*, 16: 61, 1973.
18. Ishizaka, K. and Ishizaka, T., *Asthma, Physiology, Immunopharmacology and Treatment*. Edited by K. F. Austen and L. M. Lichtenstein, p. 55, Academic Press, New York, London, 1973.