

IMMUNOGENIC PROPERTIES OF MODIFIED ANTIGEN E

I. Presence of Specific Determinants for T Cells in Denatured Antigen and Polypeptide Chains¹

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Denaturation of antigen E in 8 M urea or by reduction-carboxymethylation treatment resulted in loss of antigenic determinants in native molecules. The denatured antigen preparations, as well as the α and β polypeptide chains isolated from the antigen upon its dissociation failed to combine with rabbit or human IgG antibody against the native antigen, or to induce erythema wheal reactions in ragweed-sensitive individuals. The denatured antigens and the polypeptide chains, however, enhanced anti-DNP antibody response of DNP-ragweed (DNP-Rag)-primed rabbit lymph node cells to DNP keyhole limpet hemocyanin (KLH). When the primed lymph node cells were treated with DNP-KLH for 24 hr, and then cultured in the presence of one of these modified antigens, both IgG and IgE anti-DNP antibodies were formed in vitro. The activity of the modified antigens to stimulate carrier-specific helper cells was comparable to that of native antigen. All of the modified antigens induced DNA synthesis of peripheral lymphocytes of ragweed-sensitive patients but not the lymphocytes of normal individuals. The results indicated that denatured antigen E as well as α and β polypeptide chains possess determinants specific for T cell receptor.

The participation of two distinct lymphoid cells, i.e., thymus-derived (T)³ cells and bone marrow-derived (B) lymphocytes, in the development of humoral immune responses to a variety of antigens has been established in the mouse (1, 2). An analogous cooperation between two functionally different lymphocytes was

demonstrated in the rabbit and guinea pigs (3) in the formation of anti-hapten antibodies. It is generally accepted in the anti-hapten antibody response that precursors of antibody-forming cells, i.e., B cells, have receptors specific for hapten, and T helper cells are specific for carrier. The specificity of T cell receptor for some protein antigens appeared to be different from the specificity of B cell receptor. Using glucagon, a polypeptide of 29 amino acids, Senyk *et al.* (4) have demonstrated that antibody against the whole glucagon molecule had a specificity primarily for the amino-terminal heptadecapeptide (NM), and showed little or no binding with the carboxy terminal dodecapeptide (C). When antigen-primed lymph node cells were stimulated with the fragments, however, the C fragment induced blast transformation and synthesis of DNA, while NM peptide failed to do so.

Since the collaboration between carrier-specific helper (T) cells and hapten-specific precursors (B cells) is required for maximal anti-hapten IgE antibody response (5-7), the present

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³Abbreviations used in this paper: T, thymus derived; B, bone marrow derived; DNP-Rag, dinitrophenylated derivatives of Fr. D of ragweed antigen; DNP-KLH, DNP-keyhole limpet hemocyanin; RC, reduced carboxymethylated; UD, urea denatured; HSA, human serum albumin; Asc, *Ascaris* extract; HGG, human IgG; RGG, rabbit IgG; PCA, passive cutaneous anaphylaxis; BBS, balanced salt solution.

experiment was undertaken to study whether the T cell specificity of ragweed antigen E is different from its B cell specificity. Antigen E, the major allergen in ragweed pollen extract (8) is a labile antigen and loses its ability to induce allergic reactions on extensive chemical modification (9). Antigen E is irreversibly denatured in 8 M urea because of its dissociation into two polypeptide chains α and β (10). None of the urea-denatured (UD) antigen E and its polypeptide chains induced allergic reactions in ragweed-sensitive individuals or combined with rabbit antibody against the native antigen. The denatured antigen was found to be a poor immunogen in the rabbit. Essentially no antibody against antigen E was produced by immunization with the denatured antigen, indicating that the denatured antigen does not prime B cells specific for the native antigen. If the specificity of T cell receptor is different from the specificity of B cell receptor, however, the possibility still remains that the denatured antigen may react with T cells.

In our previous studies on anti-hapten IgE antibody formation *in vitro* by rabbit lymph node cells (11), it was shown that dinitrophenylated derivatives of Fr D of ragweed antigen (DNP-Rag) is an excellent immunogen to produce anti-DNP IgE antibody response. The mesenteric lymph node cells of rabbits primed with DNP-Rag formed anti-DNP IgE and IgG antibodies upon stimulation with the homologous antigen. Furthermore, we have succeeded in the induction of the antibody responses by stimulation of the primed lymph node cells with a mixture of DNP-keyhole limpet hemocyanin (DNP-KLH) and antigen E (12). It appears that antigen E was a carrier in this system and that the antigen stimulated carrier-specific helper cells to form soluble factor(s) which enhanced anti-DNP antibody response of hapten-primed cells. These findings provided a method to examine the ability of the carrier to stimulate carrier-specific (T) helper cells. In the present experiment, therefore, the experimental system was applied to study whether denatured antigen E and its polypeptide chains maintain the ability to stimulate carrier-specific helper cells. The results will show that these modified antigens possess immunogenic determinant(s) for helper cells.

MATERIALS AND METHODS

Antigens. Fraction D (Rag) of ragweed pollen

extract was prepared by the method of King *et al.* (8) from the low ragweed pollen (Green Laboratories, Inc., Lenoir, N. D.). Antigen E was isolated from ragweed pollen extract by the ammonium sulfate gradient solubilization procedure (13). A portion of antigen E used in the present experiment was obtained from The National Institutes of Health, Bethesda, Md. The preparation was labeled with ^{125}I (Cambridge Nuclear Corp., Billerica, Mass.) by the method of Yagi *et al.* (14) and used for radioimmunoassay. Modification of antigen E⁴ was carried out as described in (10). The antigen was denatured in 8 M urea at pH 7.95. Reduced-carboxymethylated antigen E(RC) was prepared by reduction of the protein with 0.03 M dithiothreitol in 8 M urea followed by alkylation with iodoacetic acid. Both UD and RC antigens were separated from native protein by gel filtration through a Sephadex G-100 column. The two polypeptide chains α and β of antigen E were separated by gel filtration through a Sepharose 6B column which was equilibrated with 6 M guanidine hydrochloride containing 0.025 M acetate buffer, pH 4.75. The purity of the polypeptide chains was examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (10).

KLH was purified from the blood of Limpets, (Pacific Bio-Marine Supply Co., Venice, Calif.) by the method of Campbell *et al.* (15). An extract of *Ascaris suum* was prepared and fractionated by diethylaminoethyl cellulose column as described in a previous article (12). Crystalline human serum albumin (HSA) was purchased from Nutritional Biochemical Corp., Cleveland, Ohio. Dinitrophenyl derivatives of ragweed Fr. D, *Ascaris* extract (Asc), KLH, and HSA were prepared as described in previous articles (11, 12). The number of DNP groups per milligram of DNP-Rag, DNP-Asc and DNP-KLH was 2.3×10^7 mole, 2.0×10^{-7} mole and 7×10^{-7} mole, respectively. The DNP-HSA contained 20 DNP groups per molecule.

Antibodies. Rabbit antiserum specific for antigen E was obtained by the immunization

⁴ There are two major isoelectric forms of antigen E, designated as B and C and these two forms have indistinguishable immunologic properties (9). The isoelectric difference of the two forms of antigen E was shown to be restricted to the β -chain of the molecule (10). In the present studies, the B form of antigen E was mainly used.

schedule described in a previous article (8). The antiserum gave a single precipitin band of identical specificity against ragweed extract and antigen E. Serum of a treated ragweed-sensitive patient was obtained from Dr. L. M. Lichtenstein, Johns Hopkins University, and was used as a source of IgG antibody against antigen E.

Specifically purified guinea pig anti-rabbit γ -chain antibody was the same preparation as that employed in a previous study (6). The purified antibody was labeled with ^{125}I (high specific activity, Cambridge Nuclear Corp.) by the method of Klinman and Taylor (16) and used for radioimmunoassay. Anti-DNP IgG antibody was purified from rabbit antiserum against DNP-bovine γ -globulin as described in a previous article (17).

Rabbit antiserum specific for human IgG (HGG) was prepared by immunization of rabbits with the Fc fragment of HGG included in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The antiserum rendered specific for IgG by absorption with the Fab fragment of HGG. The goat antiserum against rabbit IgG (RGG) was purchased from Antibody Inc., Davis, Calif.

Radioimmunoassay. The presence of native antigen E determinants in the modified antigen preparation was examined by evaluating the ability of these samples to inhibit the binding of ^{125}I -antigen E with either homologous rabbit or human antibody. As a preliminary experiment, a dose of rabbit antiserum or a serum of a ragweed-sensitive patient sufficient to combine 100 ng/ml of ^{125}I -antigen E was determined. One-tenth-milliliter of serial dilutions of the antisera in 300 $\mu\text{g/ml}$ of either normal RGG or HGG was incubated with an equal volume of 100 ng/ml of ^{125}I -antigen E for 4 hr, and total IgG (RGG or HGG) in the mixtures was precipitated with goat anti-RGG or rabbit anti-HGG. Measurement of radioactivity of the precipitates indicated that a 1:800 dilution of the rabbit antiserum or a 1:150 dilution of the human serum was sufficient to combine 90% of ^{125}I -antigen E. One-tenth milliliter of serial dilutions of purified antigen E (25 ng/ml to 400 ng/ml) or appropriate concentrations of modified antigens was incubated in duplicate with an equal volume of a 1:800 dilution of rabbit antiserum or a 1:150 dilution of the patient's

serum. Control tubes did not contain antigen. After 1 hr at room temperature, 0.1 ml of 100 ng/ml of ^{125}I -antigen E was added to each tube, which was kept for 4 hr at room temperature. An appropriate dose of anti-HGG or anti-RGG serum was added to each tube to precipitate all IgG in the mixture and the tubes were kept at 4°C overnight. The precipitates were washed three times with cold borate-buffered saline and their radioactivity was measured in automatic gamma counter (Nuclear Chicago Corp., North Chicago, Ill.). The quantity of antigen E determinants remaining in the modified antigens was estimated by interpolating radioactivity of the precipitates into standard curves.

Antibody response in vitro. New Zealand albino rabbits weighing 3 to 3.5 kg were primed by an i.p. injection of 10 μg of DNP-Rag included in 10 mg aluminum hydroxide gel (18), and were boosted with the same dose of antigen 4 weeks after the priming. The rabbits showing anti-DNP IgE antibody response were sacrificed 2 weeks after the booster injection to obtain mesenteric lymph nodes. The method of lymphocyte culture for antibody formation was exactly the same as that described in a previous article (6). The culture medium was enriched with 20% fetal calf serum, 2 mM L-glutamine, and 100 units/ml each of penicillin and streptomycin (Microbiological Associates, Bethesda, Md.). All of the cultures were set up in duplicate. Briefly, 2 to 3 $\times 10^7$ lymph node cells were stimulated with 0.1 $\mu\text{g/ml}$ of DNP-Rag or DNP-KLH. After incubation at 37°C for 24 hr, the cells were washed and 1 to 2 $\times 10^7$ nucleated cells suspended in 1 ml of the culture medium were cultured by the method of Marbrook (19) for 6 days in a humidified incubator with a gas flow of 5% CO_2 and 95% air. In order to examine the effect of free-carrier on the antihapten antibody response to DNP-heterologous carrier conjugate, we treated aliquots of the DNP-Rag-primed lymph node cells with 0.1 $\mu\text{g/ml}$ of DNP-KLH for 24 hr. After washing, the cells were cultured for 6 days in the culture medium containing an appropriate concentration of either antigen E or modified antigen (*c.f.*, 12).

Measurement of antibodies. Anti-DNP IgG antibody in the cell culture fluids was measured by radioimmunoassay with Sephadex-immunosorbent coupled with DNP-HSA and ^{125}I -labeled anti- γ -chain antibody. The method

was described in detail in a previous article (20). Anti-DNP IgE antibody was determined by the passive cutaneous anaphylaxis (PCA) reaction in the rabbit with DNP-HSA as challenging antigen (17).

Measurement of ^3H -thymidine uptake by human lymphocytes. Heparinized blood was obtained from both ragweed-sensitive hay fever patients and normal individuals, and mononuclear cell fraction was obtained by the method of Böyen (21). One volume of the blood was diluted with 3 volumes of Hanks' balanced salt solution (BBS) (Microbiological Associates) and 30 ml of the cell suspension were layered on top of 10-ml Hypaque-Ficoll layer with a density of 1.077. The tubes were centrifuged at $400 \times G$ for 20 min at room temperature and the layer of mononuclear cells was recovered. The cells were washed three times with Hanks' BBS, and the cell pellet was resuspended in culture medium (RPMI 1640, Microbiological Associates) enriched with 10% human type AB serum and 2 mM L-glutamine. One-tenth milliliter of antigen to be tested on culture medium was added to 1.0 ml of the cell suspension containing 2×10^6 mononuclear cells. After mixing, 0.2 ml each of the cell suspension was delivered into four polyethylene microfuge tubes (Beckman Instrument Inc., Fullerton, Calif.) and cultured at 37°C in a humidified incubator with a gas flow of 5% CO_2 and 95% air. After 5-days culture, 1 μCi of ^3H -thymidine (1.9 Ci/mmol specific activity, Schwarz/Mann, New York, N. Y.) was added to each tube and incubated for another 24 hr. All the cells in each tube were harvested on a glass filter (1 cm diameter, Whatman glass filter paper 6F81) and washed by suction with 10 ml cold saline, 5 ml of cold trichloroacetic acid and finally with 1 ml absolute methanol. The filter paper was dried by incubation at 37°C for 30 min, immersed in 10 ml Bray's solution, and radioactivity was measured in an automatic Tri-Carb liquid scintillation spectrometer (Packard Instrument Co., Downer's Grove, Ill.).

RESULTS

As a preliminary experiment, modified antigen E preparations were tested for the ability to react with the antibody against native antigen. In immunodiffusion against antiserum specific for antigen E, none of the UD, RC antigens,

α -chain and β -chain preparations gave a precipitin band at a concentration of 1 mg/ml, whereas 20 $\mu\text{g}/\text{ml}$ of antigen E did. In order to detect possible contamination of native antigen in the modified antigen preparations, we tested 100 $\mu\text{g}/\text{ml}$ of modified antigens for ability to inhibit the binding of ^{125}I -labeled antigen E with human and rabbit antibodies against antigen E. In the rabbit antibody system, preincubation of 100 ng/ml of cold antigen E with a 1:800 dilution of the antiserum diminished the binding of ^{125}I -antigen E with the antibody by 13%, but none of the modified antigens, i.e., UD, RC antigens, or α - and β -chain, at the level of 100 $\mu\text{g}/\text{ml}$, showed a significant inhibition. When a 1:150 dilution of patient's serum was used in the test, 25 ng/ml, 50 ng/ml, and 100 ng/ml of cold antigen E diminished the binding of ^{125}I -antigen E by 12%, 29%, and 59%, respectively. The binding activity of 100 $\mu\text{g}/\text{ml}$ of UD antigen was comparable to that of 35 ng/ml of antigen E, however, the activity present in 100 $\mu\text{g}/\text{ml}$ of RC antigen, α -chain and β -chain was less than that of 25 ng/ml of antigen E. The results indicated that native antigenic determinant present in the preparations of modified antigens was less than 0.04% of the native antigen on a weight basis.

The ability of the modified antigens to stimulate carrier-specific helper cells was studied in the antibody response *in vitro*. The mesenteric lymph node cells were obtained from the rabbits primed with DNP-Rag. Five duplicate sets of the cell suspension were incubated in 0.1 $\mu\text{g}/\text{ml}$ of DNP-KLH. After 24 hr, the cells were washed and each set of the DNP-KLH-treated cells was resuspended in culture medium containing untreated antigen E or one of the modified antigens. The concentration of the antigen in the cell suspension was 10 $\mu\text{g}/\text{ml}$. One set of the DNP-KLH-treated cells was cultured without the addition of antigen. As a control, the primed lymph node cells were stimulated with 0.1 $\mu\text{g}/\text{ml}$ of DNP-Rag for 24 hr and then cultured in fresh medium. As shown in Figure 1, the addition of native antigen E to DNP-KLH-treated cells significantly enhanced the anti-DNP antibody responses of IgG and IgE classes. The results showed that all of the UD antigen, RC-antigen, α -chain and β -chain enhanced the anti-hapten antibody response, comparable to that obtained with untreated antigen E. In

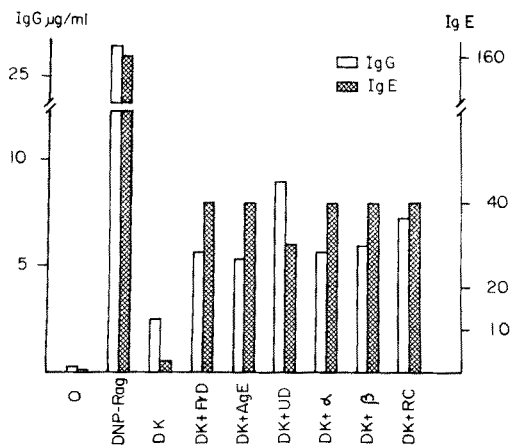


Figure 1. Anti-DNP antibody response of DNP-Rag-primed rabbit lymph node cells. Mesenteric lymph node cells were incubated in 0.1 µg/ml of DNP-Rag or DNP-KLH(D-K) for 24 hr. After washing, cells stimulated with DNP-Rag were cultured in fresh medium. Cells treated with DNP-KLH were cultured in the presence (10 µg/ml) of Fr. D, UD antigen, α-chain, β-chain, or RC antigen. Aliquots of DNP-KLH treated cells were cultured in fresh medium as a control.

order to prove that carrier-specific helper cells are required for the enhancement of the antibody response by antigen E, we studied the effect of the antigen with mesenteric lymph node cells of a rabbit immunized with DNP-Asc. As reported in a previous article (12), anti-DNP antibody response of the DNP-Asc-primed cells to DNP-KLH was enhanced by the addition of free Asc but not by Fr. D. It was also found that the addition of native antigen E or UD antigen did not enhance the antibody response of the DNP-Asc primed cells to DNP-KLH (data not shown).

The possibility remained that the enhancing effect of the modified antigens might be due to contamination of the preparations with native antigen. For studying this possibility, 10-fold dilutions of antigen E and modified antigens were employed to stimulate carrier-specific cells. The mesenteric lymph node cells from DNP-Rag-primed animals were incubated in 0.1 µg/ml of either DNP-Rag or DNP-KLH for 24 hr. After washing, duplicate sets of the DNP-KLH-treated cells were cultured in fresh medium containing 0.1 µg/ml, 1.0 µg/ml, or 10 µg/ml of antigen E or α-chain, or in the medium containing 10 µg/ml of β-chain. Aliquots of the

DNP-KLH-treated cells were cultured without the addition of antigen. The results of the experiment are shown in Table I. Since IgE antibody formed *in vitro* by the stimulation with DNP-Rag was low in one experiment (No. 2), only IgG antibody was shown. The results indicated that both α-chain and β-chain enhanced IgG and IgE anti-hapten antibody responses. Considering that variation in antibody concentration in the duplicate cultures was 10 to 20%, enhancing effect of α-chain was comparable to that of native antigen E. In one experiment (No. 3), β-chain appeared to be less active than native antigen with respect to the enhancement of antibody response, however, the effect of β-chain could not be explained by possible contamination of the preparation with undenatured molecule.

The activity of modified antigens to induce DNP synthesis of human lymphocytes was studied. Mononuclear cell fraction of peripheral blood was obtained from both ragweed-sensitive

TABLE I
Enhancement of anti-DNP-antibody response by antigen E and modified antigens

Antigen for Stimulation <i>in vitro</i>		Anti-DNP Antibodies Formed		
		Rabbit no. 2	Rabbit no. 3	
0 hr ^a	24 hr	IgG	IgG	IgE ^b
	µg/ml	µg/ml	µg/ml	
None	None	0.27	1.0	5
DNP-Rag	None	36.0	35.0	80
DNP-KLH	None	3.4	5.0	5
AgE				
DNP-KLH	0.1	5.4	7.8	5
DNP-KLH	1.0	5.3	11.0	10
DNP-KLH	10	10.5	13.0	15
α-chain				
DNP-KLH	0.1	3.7	7.5	5
DNP-KLH	1.0	7.1	9.0	10
DNP-KLH	10	11.0	11.5	15
DNP-KLH	β-chain 10	12.0	10.0	10

^a DNP-Rag-primed lymph node cells were incubated with DNP-carrier conjugate. After 24 hr, cells were washed and cultured in fresh medium, some of which contained an appropriate concentration of ragweed antigen.

^b PCA titer.

patients and European non-atopic individuals who consequently had never been exposed to ragweed pollen. The cells were incubated with varying concentrations of antigen E or modified antigens. The same cell suspension was cultured without antigen for the same period of time as a control. ^3H -thymidine uptake of the antigen-stimulated cells and unstimulated cells was determined. Experimental error among the four tubes derived from the same stimulated cell suspension was less than $\pm 10\%$. Average radioactivity of the stimulated cells was divided by the radioactivity of unstimulated cells and the ratios are shown in Table II. It is apparent that all of the modified antigens stimulated DNA synthesis of lymphocytes from ragweed-sensitive individuals. In some cases, such as GS, UD, and RC antigens were slightly less active than native antigen, however, the activity of the denatured antigens could not be explained by contamination with native antigen.

DISCUSSION

It is well established that the determinants of ragweed antigen E to bind with the specific IgG or IgE antibodies depend on the conformation of the antigen (8, 10). As reported previously (10)

and confirmed in the present experiment, none of the UD antigen, RC antigen, and the two polypeptide chains isolated from the antigen induced positive erythema wheal reactions in ragweed-sensitive individuals (results not shown), or combined with rabbit or human IgG antibody against the native antigen. The present experiment, however, clearly showed that the denatured antigens, as well as the polypeptide chains, enhanced anti-DNP antibody response of DNP-Rag-primed rabbit lymph node cells to DNP-KLH. The effect of the antigen was specific. The anti-DNP antibody response to DNP-KLH of the lymph node cells primed with DNP-Asc was not enhanced by the addition of UD antigen. In view of the accumulated evidence that the enhancement of anti-DNP antibody response to DNP-KLH by free carrier is due to the release of enhancing soluble factor from helper cells (12), it appears that modified antigens, which have no antigenic determinants for humoral antibody, were capable of stimulating helper cells. The origin of carrier-specific helper cells is not yet established in the rabbit. Functionally, however, these cells correspond to T cells in mice. Under the present experimental condition, an optimal concentration of free

TABLE II
Stimulation of human peripheral lymphocytes by modified antigen E for ^3H -thymidine uptake^a

Donor of Lymphocytes	Antigen Concn	Antigen for Stimulation				
		Untreated E	Urea	α -chain	β -chain	RC
	$\mu\text{g/ml}$					
Patient ^b						
GS	10	16.6	8.1	ND ^c	16.3	8.1
	1	4.6	5.4	ND	4.6	2.6
GS	10	13.1	5.7	13.3	10.8	ND
GC	10	6.0	5.4	6.3	5.3	ND
	1.0	3.2	3.0	3.0	2.6	ND
	0.1	1.4	1.6	2.0	1.3	ND
ML	10	5.7	4.0	4.8	4.8	4.7
	1.0	1.9	3.2	2.6	2.2	2.8
	0.1	1.2	1.5	1.9	ND	ND
Normal						
GD	10	0.8	0.8	0.9	1.1	0.8
WK	10	1.2	1.2	1.0	1.0	1.0

^a Numbers in this table represent ratio of thymidine incorporation in cultures with antigen present relative to cultures without antigen from the same cell suspension.

^b Ragweed-sensitive hay fever patients.

^c ND, not done.

carrier to stimulate helper cells for the release of enhancing soluble factor was 10 $\mu\text{g/ml}$, whereas 0.1 to 1 $\mu\text{g/ml}$ of Fr. D was optimal for maximal anti-carrier antibody response (11). Furthermore, our more recent studies, which will be published separately, showed that cellular immunosorbent coated with anti-rabbit Fab antibody removed DNP-specific precursors, but failed to remove the carrier-specific cells which respond to the high concentration (10 to 100 $\mu\text{g/ml}$) of free carrier for the induction of thymidine uptake. It is generally accepted that precursor cells have immunoglobulin receptors, of which specificity is identical to that of humoral antibody produced by their progeny (22, 23). Since the antigenic determinant for humoral antibody is lacking in the modified antigens, it is apparent that the cells stimulated by the modified antigens and releasing enhancing soluble factor were not antigen E-specific precursor (B) cells.

It has been shown by Girard *et al.* (24) and Brostoff *et al.* (25) that peripheral blood lymphocytes from hay fever patients are often transformed by allergens. The present data confirmed and extended these observations through the demonstration that modified antigen E, which is incapable of binding with humoral antibody, induced DNA synthesis of peripheral lymphocytes of ragweed-sensitive individuals. In humans, the majority of peripheral lymphocytes which undergo blast transformation in response to antigenic stimulation is believed to be T lymphocytes (26). The induction of blast transformation by anti-heavy chain antibodies (27) suggests that stimulation of B cells can also result in increased thymidine uptake. Lack of antigenic determinants in the modified antigen E, however, strongly suggests that these antigens actually stimulated antigen-specific T cells. Thus, it appears that the denatured antigen E as well as α - and β -polypeptide chains possess determinants specific for T cell receptor.

Senyk *et al.* (4) as well as Alkan *et al.* (28), demonstrated that specificity of glucagon and L-tyrosine-azobenzene-arsenate (RAT)-poly- γ -D-glutaminic acid (PGA) conjugates for T cells are different from the determinants for B cells. It appears that the same principle is applicable to allergens and the immunocompetent cells participating in the IgE antibody response. In our

previous article (11), we have shown evidence that carrier-specific helper cells involved in anti-hapten IgE antibody response (IgE-T cells) are different from the helper cells for IgG antibody response (IgG-T cells). Enhancement of both IgE and IgG anti-DNP antibody responses by the polypeptide chains of antigen E suggests that receptors on IgE-T cells and IgG-T cells have a common specificity.

Stimulation of antigen E-specific T cells by modified antigens may have some practical importance. In our previous studies on the effect of immunotherapy of ragweed-sensitive hay fever patients, we have shown evidence that IgE antibody against ragweed allergen was significantly diminished in more than one-half of treated patients (29). As the mechanisms for the decrease of IgE antibody level did not appear to be due to feed back regulation by an increased IgG antibody, we have speculated on the possibility that a population of immunocompetent cells may have changed by the repeated injections of allergen. Since modified antigens which do not induce allergic reactions in the patients are capable of stimulating T cells, one might speculate that injections of such modified antigens may change the T cell population without side effects. For analyzing the mechanisms of immunotherapy and applying the present findings in atopic individuals, further studies on modified antigens are required. Experiments are now in progress to study the ability to UD antigen and α -chain to prime antigen E-specific T cells in inbred strains of mice.

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