

HLA-LINKED NONRESPONSIVENESS TO CRYPTOMERIA JAPONICA POLLEN ANTIGEN

I. Nonresponsiveness Is Mediated by Antigen-Specific Suppressor T Cell¹

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The objective of this study was to elucidate the cellular mechanism of IgE nonresponse to the *Cryptomeria japonica* (Japanese cedar) pollen antigen (CPAg), which was shown in our previous study to be HLA-linked (1). We established an assay system for the measurement of small amounts of anti-CPAg IgE antibody, both in an antigen-specific and isotype-specific manner, and a culture system to induce antigen-driven IgE antibody synthesis in vitro. By using these methods, we clarified that the function of the HLA-DR molecule in the CPAg-driven IgE response is similar to that of I-A or I-E molecule in mice, namely the product of immune response genes (*Ir-genes*), because anti-HLA-DR monoclonal antibody blocked the response, and the interaction between monocyte and monocyte-depleted peripheral blood lymphocytes (PBL) to respond to CPAg was restricted by HLA-DR. Furthermore, PBL from nonresponders revealed a specific IgE response to CPAg when the Leu-2⁺3⁻ T cell fraction was depleted, thereby suggesting that even nonresponders have Leu-2⁺3⁺ T cell and B cell clones specific for CPAg, and they apparently show no response due to the presence of CPAg-specific Leu-2⁺3⁻ suppressor T cells. This suppressor T cell fraction abolished the IgE response of the autologous B + monocyte + Leu-2⁺3⁺ T cell in a CPAg-specific manner. The current cellular analysis together with our previous genetic analysis strongly suggest that the HLA-linked IgE nonresponse to CPAg is mediated by CPAg-specific suppressor T cells. The HLA-linked gene controlling the nonresponsiveness to CPAg is thus designated as the immune suppression gene for CPAg (*Is-CPAg*).

Mapping of *Is-CPAg* within HLA-DQ subregion is discussed.

We have already demonstrated that the low responsiveness to streptococcal cell wall (SCW) antigen is an HLA-linked single dominant trait (1, 2). Even monocytes from low responders presented SCW antigen to T cells of high responders when they shared at least one HLA-DR antigen, and low responders had antigen-specific suppressor T cells. Thus the low responsiveness to SCW is controlled by the HLA-linked immune suppression gene "*Is-SCW*" through antigen-specific suppressor T cells (3).

Allergy seems to be under the control of genetic factors as determined in a study on twins (4), association with HLA (5), and a linkage study (6). Analyses of cellular mechanisms of these antigen-specific IgE responses have been hampered because of difficulties in the induction of specific IgE antibody response in vitro (7-23) and in assay systems for detection of minute amounts of IgE (24-26).

We carried out a genetic analysis of cryptomeria pollinosis (CP),⁴ the most common type I allergy caused by cryptomeria pollen in Japan. Studies on 165 multiplex families revealed that the resistance to CP is an HLA-linked single dominant trait; in other words, susceptibility to CP is a single recessive trait (1, 2).⁵ The resistance to CP was in negative linkage disequilibrium with HLA-DQw3 ($p < 0.03$). To investigate the regulatory mechanism of IgE response to *Cryptomeria japonica* pollen-antigen (CPAg), methods for the induction of specific IgE response in vitro and for the measurement of trace amounts of antigen-specific IgE have to be established. We now report that anti-CPAg IgE antibody can be induced in vitro, and the antibody can be measured in an antigen-specific and isotype-specific manner.

MATERIALS AND METHODS

Rabbit anti-human IgE antibody. We prepared the anti-human IgE antibody by immunizing rabbits with IgE(PS) and purified the antisera by different IgE protein, ND, to rule out possible cross-reactions with the idiotype of PS (24). The anti-IgE antibody was raised in rabbits by immunizing IgE myeloma protein (PS). The

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⁴ Abbreviations used in this paper: CPAg, *Cryptomeria japonica* pollen antigen; MTag, mite antigen; SCW, Streptococcal cell wall; MAb, monoclonal antibody.

⁵ Muto, M., N. Yasuda, Y. Saito, and T. Sasazuki. 1986. Genetic Analysis of Cryptomeria Pollinosis. Submitted for publication.

antibody was made specific for epsilon chain by extensive absorption on normal IgG-column and was purified on IgE(PS)-Sephacryl and IgE(ND)-Sephacryl columns as described (23).

Anti-HLA monoclonal antibodies (MAB), Ig, and chemical reagents. Human monoclonal IgG, human myeloma IgA, human myeloma IgM, affinity-purified anti-human IgA, affinity-purified goat anti-human IgG, Fc, and anti-human IgM were purchased from Cappel Laboratories, West Chester, PA. CNBr-activated Sepharose 4B, Sephacryl S-200, and Phenyl-Sepharose were from Pharmacia Fine Chemicals, Uppsala, Sweden. DEAE cellulose and CM cellulose were from Whatman Ltd., England. MAB PA2-6, HU-series and B7/21 were kindly provided by Dr. P. Parham (Stanford University, CA), Dr. M. Aizawa (Hokkaido University, Japan), and Dr. F. Bach (University of Minnesota, Minneapolis, MN), respectively (27). The MAB HU-4 recognizes the framework of the HLA-DR molecule and did not react with the DQw1 molecule.

Gel electrophoresis, Western transfer, and immune hybridization. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 10% homogenous acrylamide gel. Crude pollen extract treated with mercaptoethanol was layered over the width of the stacking gel. By using a modification of the method of Towbin et al. (28), protein bands were transferred to a nitrocellulose membrane at a constant voltage of 150 V over an 8 cm path for 60 min with the use of a transfer blotting system (Bio-Rad, CA). The transfer buffer was 0.025 M Tris, 0.192 M glycine, pH 8.3, 20% v/v methanol, and 0.1% SDS. After transfer, a strip of the membrane was cut and was stained with amido black to check the efficiency. The membrane was then washed for 60 min in 10% methanol in 10 mM phosphate-buffered saline (PBS) to remove SDS, followed by a 15-min wash in PBS, then was shaken for 60 min in 0.1% Tween 20 in PBS (TPBS) to block the unsaturated membrane sites. The sliced membrane was then incubated with serum for 90 min at room temperature with occasional mixing, was washed with TPBS, and was incubated with ^{125} I-labeled anti-human IgE antibody. After 60-min incubation at room temperature, the strips were thoroughly washed with TPBS and were autoradiographed for 3 days.

Preparation of antigens. The major bands of CPAG were prepared as described (29) with some modifications. Briefly, dry pollen was defatted with ether, was placed in 0.125 M ammonium bicarbonate, and was stirred for 2 days at 4°C. Solid ammonium sulfate was added to the extract to 100% saturation. The precipitate was dissolved in 0.05 M Tris-HCl, pH 7.8, and was dialyzed against the same buffer. The sample was applied to a DEAE-cellulose column and was eluted with the same buffer. The eluate was dialyzed against 0.01 M acetate buffer (pH 5.0) and was applied to a CM-cellulose column. The column was eluted first with 0.01 M acetate buffer then with 0.1 M phosphate buffer, pH 7.0, and the second eluate was concentrated and was applied to a Sephacryl-S200 column equilibrated with PBS, pH 7.2. The 41,000 and 46,000 dalton proteins thus obtained were stored frozen in small aliquots and were used for cell culture and radioimmunoassay (RIA). The antigenic purity of 41,000 + 46,000 dalton proteins was the same as that of Yasueda et al. (29). Additional purification of each protein molecule was performed by hydrophobic chromatography on a phenyl-Sepharose column with a linear gradient elution from 1.5 M ammonium sulfate/0.01 M phosphate buffer, pH 6.8 to 0.01 M phosphate buffer. These single bands were used only for the purpose of checking the reactivity with patients' sera. Lyophilized crude mite extract was kindly provided by Torii & Co. Ltd., Tokyo, Japan.

Purification of anti-CPAG antibody. Saturated ammonium sulfate solution was added to pooled human sera from approximately 100 patients with CP to 50% saturation. The precipitate was dissolved in PBS, was dialyzed against PBS, and then was applied to a CPAG-coupled Sepharose 4B column equilibrated with 10 mM PBS, pH 7.2. After extensive washes, the column was eluted with 0.1 M glycine-HCl, pH 2.8. The eluate was immediately neutralized with solid trisaminomethane, was dialyzed against PBS, and was stored at 4°C with 0.02% sodium azide. Anti-CPAG IgE antibody/total IgE ratio was thus enriched 19 times (data not shown).

RIA for the determination of the cross-reactivity of anti-human IgE antibody with IgA, IgG, and IgM. Polyvinyl plates were coated for 40 hr at 4°C with 100 μ l of the mixture of anti-IgE, anti-IgA, anti-IgG, and anti-IgM antibody, with each protein concentration of 20 μ g/ml. After three washes with PBS, the wells were filled with 10% fetal calf serum (FCS)/PBS for 3 hr at 4°C to block unsaturated protein-binding sites. The plates were then washed 10 times with PBS, and 100 μ l of Ig in 10% FCS/PBS were added to each well in triplicate followed by incubation for 5 hr at 37°C. After the 10 washes with PBS, 100 μ l of anti-IgE antibody labeled with ^{125} I by using the solid-state lactoperoxidase method (20) in 10% FCS/PBS were dispensed to each well, and the preparation was allowed to react for 3 hr at room temperature. The wells were washed thoroughly with PBS and then with tap water, were cut, and their

radioactivities were counted in an automatic gamma counter.

RIA for total IgE measurement. Serially diluted IgE myeloma protein (PS) and a sample with an unknown concentration of IgE were applied to the triplicate wells precoated with 50 μ g anti-IgE antibody/ml of PBS. After incubation with 10% FCS/PBS, 100 μ l of ^{125} I-labeled anti-IgE (200 ng/ml, 20 to 50 $\times 10^6$ cpm/ μ g) in 10% FCS/PBS were added to each triplicate well followed by 3-hr incubation. The wells were washed, were cut, and were counted.

RIA for anti-CPAG IgE antibody. The procedure was the same as for the measurement of total IgE except that the wells were precoated with 100 μ l of CPAG (100 μ g/ml of PBS). Purified anti-CPAG Ab of which the total IgE concentration was determined as mentioned above, was applied to this assay system to make a standard curve for the measurement of anti-CPAG IgE Ab. Anti-mite antigen (MTAg) IgE Ab was measured by using the same procedure. The lower limit of the antibody assayed in this system was 10 to 30 pg/ml (data not shown). The SD of the triplicate assay was less than 30% at approximately 50 pg/ml and less than 15% at approximately 100 pg/ml.

HLA typing. HLA-A, B, and C typing was done by the microdroplet lymphocytotoxicity test. HLA-DR, DRw, and DQ antigens were typed by the two stage microcytotoxicity assay by using B cell-rich lymphocytes prepared by the modified thrombin nylon wool method (30). Anti-HLA antisera used were distributed for the Ninth International Histocompatibility Workshop, the Third Asia Oceania Histocompatibility Workshop, and the Ninth Japan Histocompatibility Workshop, as well as local sera.

Cell cultures. Diagnosis of CP was based on the clinical manifestation and skin test, as well as specific IgE titer in serum, and the patients were bled mid- and post-pollination season, namely, February to July. Cells were cultured to induce immune response to CPAG as described elsewhere (20) with some modifications. Three million PBL isolated from heparinized peripheral venous blood on Ficoll-Conray gradient were cultured in 12 $\times$ 75 mm polystyrene tubes (Falcon Plastic, Becton, Dickinson & Co., Oxnard, CA) in 2 ml of RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine, and 10% heat-inactivated FCS in a humidified atmosphere of 5% CO₂ at 37°C, with or without antigen and/or pokeweed mitogen (PWM). All the buffer solutions of antigens and PWM were replaced with PBS, and the same volume of solution was added to every culture to avoid the dilution effect. After 60-hr incubation, the cells were washed three times with Hanks' balanced salt solution and were resuspended in 2 ml of culture medium. The cells were then cultured in 24-well culture plates (Linbro) for an additional 6 days. A sample culture without antigens, mitogen, or cycloheximide was sonicated immediately after the wash. Culture supernatants were harvested by centrifugation (8000 $\times$ G and 350 $\times$ G for 10 min with or without sonication, respectively) and were kept at -80°C until assayed.

Cell fractionation. Peripheral blood lymphocytes (PBL) were suspended in 10 ml of 5% FCS/PBS and were placed in plastic dishes (90 $\times$ 20 mm; Nunc, Roskilde, Denmark) followed by incubation for 60 min at 37°C. The dish-nonadherent cells were harvested. After extensive washes, the dish-adherent cells were removed by scraping with a rubber policeman, and this served as a monocyte fraction. Dish-nonadherent cells were then treated with anti-Leu-M3 monoclonal antibody (Becton, Dickinson & Co., Sunnyvale, CA) and rabbit complement (31). This fraction was used as the monocyte-depleted PBL. T and B cells were separated from this fraction by E-rosetting with neuraminidase-treated sheep red blood cells. The cells not forming rosettes were collected as B cells. T cells obtained by E-rosetting from PBL or from monocyte-depleted PBL were divided into helper and suppressor/cytotoxic T cell fractions by the panning method of Wysocki and Sato (32) by using anti-Leu-2a or anti-Leu-3a monoclonal antibody (Becton, Dickinson & Co., Sunnyvale, CA). By using the anti-Leu-2a monoclonal antibody, positivity of the Leu-2 and Leu-3 antigens of the bound cells exceeded 95% and was less than 7% and more than 90%, respectively. The fractionated cell populations were co-cultured in the concentrations of 0.25 $\times 10^6$, 0.25 $\times 10^6$, 0.5 $\times 10^6$, and 0.5 $\times 10^6$ /ml for monocytes, B cells, Leu-2⁺3⁺ T cells and Leu-2⁺3⁻ T cells, respectively.

Cell culture with MAB. The ascites containing MAB or the control ascites without MAB were added to the culture at the final dilution of 1/1,000, both at the initiation of the culture and after the wash on day 3. As a control, we used ascites of BALB/c mouse injected i.p. with the murine myeloma cell line P3-X63-Ag8. We confirmed by indirect immunofluorescence staining that the titer of PA2-6, B7-21, HU-11, and HU-4 was 1: $>10,000$, 1:3,000, 1:10,000, and 1:10,000, respectively.

Cell culture with allogeneic monocytes. Three million monocyte-depleted PBL were co-cultured with 0.3 $\times 10^6$ of dish-adherent cells

of autologous or allogeneic origin in 2 ml of culture medium with PWM and/or CPAG.

RESULTS

Identification and purification of major allergens of *cryptomeria japonica* pollen. The major allergens of *cryptomeria japonica* pollen were identified by the immunoblot technique as shown in Figure 1. The crude ammonium bicarbonate extract revealed 13 bands on SDS-PAGE (Lane 1). All of these bands were electrophoretically transferred to a nitrocellulose membrane, and an aliquot was stained with amido black to check the efficiency (Lane 2). The sliced membrane was then incubated with the mixture of sera from 10 individuals with CP or with sera from non-CP individuals (Lanes 3 and 4). The allergens were detected by the following incubation with anti-human IgE antibody labeled with ^{125}I and autoradiography. The 41,000 and 46,000 dalton proteins showed the strongest reaction with the patients' sera. A fraction of the 41,000 dalton protein plus the 46,000 dalton protein purified as described in *Materials and Methods* was used in the culture and the RIA, because these proteins well reflect the total allergenicity of crude extract from *cryptomeria* pollen as we have already observed and as in another report (29); every patient reacted with both (anti-41,000 protein IgE Ab/anti-46,000 protein IgE Ab ratio measured in RIA ranged from 0.4 to 1.3 by using sera from 16 patients; data not shown); and the two molecules are indistinguishable in amino acid constitution (29).

Reactivity of anti-human IgE antibody with IgA, IgG, and IgM. The levels of cross-reactivity of anti-IgE Ab with other Ig isotypes were scrutinized by solid-phase RIA. Serial dilutions of IgE(PS), myeloma IgA, polyclonal IgG, and myeloma IgM protein were added to wells pre-coated with the mixture of anti-IgE, anti-IgA, anti-IgG, and anti-IgM Ab with the concentration of 20 $\mu\text{g}/\text{ml}$ of each protein, and ^{125}I -labeled anti-IgE Ab was added. Table I shows that the IgE protein revealed high reactivity with anti-IgE Ab (924 cpm in 0.1 ng/ml and 6315 cpm in 1.0 ng/ml), whereas other Ig isotypes revealed a trace reactivity with anti-IgE Ab, even at the concentration of 100 $\mu\text{g}/\text{ml}$. Only IgA seemed to show visible binding, but

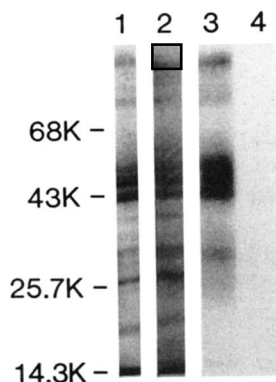


Figure 1. Western immunoblot profile. A mixture of ten patients' sera was incubated with a nitrocellulose membrane on which CPAG separated on 10% SDS-PAGE was electrophoretically transferred. CPAG-anti-CPAG antibody complexes were incubated with ^{125}I -anti-human IgE antibody and were autoradiogrammed. Lane 1, crude extract on 10% SDS-PAGE stained with Coomassie Brilliant Blue. Lane 2, transferred bands on nitrocellulose membrane stained with amido black. Lane 3, mixture of 10 patients' sera, autoradiogrammed. Lane 4, mixture of 10 non-CP sera, autoradiogrammed.

TABLE I
Isotype specificity of anti-human IgE antibody

Sample	Concentration of Sample (ng/ml)	Median cpm
IgE (PS)	BKG	591
	0.03	673
	0.10	924
	0.30	2012
	1.0	6315
IgA	BKG	577
	10^2	669
	10^3	819
	10^4	860
	10^5	902
IgG	BKG	580
	10^2	602
	10^3	590
	10^4	598
	10^5	621
IgM	BKG	550
	10^2	602
	10^3	629
	10^4	626
	10^5	647

^a Each sample was applied to wells precoated with the mixture of anti-IgE, anti-IgA, anti-IgG, and anti-IgM, and then ^{125}I -anti-IgE antibody was added.

TABLE II
Antigen specificity of the assay system^a

Serum Donor	Skin Test		IgE in Serum ^b	
	CPAg	MTAg	Anti-CPAg	Anti-MTAg
IN	+	+	24,305	14,747
HY	+	-	21,105	-26
GY	-	+	64	11,510
TI	-	-	46	31

^a Sera with various skin-testing patterns were added to wells precoated with CPAG or MTAg.

^b Expressed in cpm = median cpm - background cpm (FCS as background).

the reaction of 100 $\mu\text{g}/\text{ml}$ of IgE (924 cpm) corresponds to 1 million-fold IgA protein (100 $\mu\text{g}/\text{ml}$, 902 cpm). Taking into account the finding that the specific IgA Ab induced in vitro never exceeded 1 $\mu\text{g}/\text{ml}$, the cross-reactivity observed here can probably be disregarded. Furthermore, the cross-reactivity with light chains is ruled out because the IgG we used here is polyclonal.

Antigen specificity in RIA. The serum from donor IN, who revealed positive skin tests both to CPAG and to MTAg, showed high reactivity both with CPAG and MTAg. The sera from HY who is allergic to CPAG but not to MTAg showed high reactivity with CPAG, whereas the reactivity with MTAg was the same as the background count. The antigen specificity in the assay was also confirmed by using sera from a donor GY who is allergic to MTAg but not to CPAG (Table II). These results indicate that contamination of nonspecific IgE Ab was ruled out in the assay for specific IgE Ab. From the results shown in Tables I and II, we concluded that anti-CPAG IgE Ab can be measured in an IgE-specific and in an antigen-specific manner.

Induction of specific IgE antibody production in vitro and cell fractions required for the response. PBL from a donor allergic to CPAG were incubated with PWM with or without CPAG or cycloheximide. As shown in Table III, the CPAG-specific IgE responses of patients in vitro are divided into three categories; patients who show no enhanced anti-CPAG IgE Ab with PWM in vitro (donor MY),

TABLE III
De novo specific IgE response in vitro and its cell-fraction dependence^a

Cell Fraction ^b	CPAg ^c	PWM	Cycloheximide	Anti-CPAg IgE (pg/ml)
PBL (MY)	—	+	—	*
	3	+	—	*
	10	+	—	*
	30	+	—	*
PBL (KH)	—	+	—	95
	3	+	—	50
	10	+	—	70
	30	+	—	100
PBL (HS)	—	+	—	200
	3	+	—	140
	10	+	—	145
	30	+	—	40
PBL (IU)	—	+	—	*
	10	+	—	55
	30	+	—	90
PBL (KU)	—	+	—	30
	3	+	—	65
	10	+	—	105
	30	+	—	*
PBL (RO)	—	+	—	70
	3	+	—	325
	10	+	—	135
	30	+	—	55
PBL (SM)	—	—	—	*
	—	+	—	40
	10	—	—	*
	10	+	—	90
	10	+	+	*
	sonicated after wash ^d			30
B + Mo (SM)	10	+	—	30
T	10	+	—	*
B + Mo + Leu-3 ⁺ T	10	+	—	125
B + Mo + Leu-2 ⁺ T	10	+	—	*
B + Leu-3 ⁺ T	10	+	—	40

^a PBL from a patient with CP was cultured with CPAg, and/or PWM (1 µg/ml) and/or cycloheximide (100 µg/ml).

^b Panning was performed by anti-Leu-2a MAb.

^c Expressed in µg/ml.

^d PBL were sonicated immediately after washing on day 3. *, <30 pg/ml.

patients who show an enhanced anti-CPAg IgE Ab with PWM but show no additional enhancement by addition of CPAg to culture (donors KH and HS), and patients who show a higher anti-CPAg IgE Ab with PWM plus CPAg than with PWM only (donors IU, KU, RO, and SM). By using donor SM, significant IgE Ab production was induced when the cells were cultured with PWM and CPAg (IgE Ab = 90 pg/ml). The level of specific IgE Ab in culture supernatants of PWM plus CPAg was significantly higher than that of PWM only (IgE Ab = 40 pg/ml). In addition, production of the specific IgE Ab was derived from de novo synthesis, in that it was inhibited by the protein synthesis inhibitor, cycloheximide (IgE Ab < 30 pg/ml), and sonication of the PBL had a low level of the antibody (IgE Ab = 30 pg/ml). Furthermore, the significant specific IgE response was observed when B cells, monocytes, and Leu-2⁺3⁺ T cells were cultured with CPAg plus PWM (IgE Ab = 125 pg/ml). On the contrary, the response was not observed when the culture was performed without T cells (IgE Ab = 30 pg/ml), without monocytes (IgE Ab = 40 pg/ml), or with Leu-2⁺3⁺ T cells instead of Leu-2⁺3⁺ T cells (IgE Ab < 30 pg/ml). These results indicate that the antigen-driven de novo CPAg-specific IgE response occurs in vitro and the response is B cell-, monocyte-, and Leu-2⁺3⁺ T cell-dependent.

Effect of MAb on specific IgE response. In patients who revealed the antigen-driven specific IgE Ab production as shown in Table III, we studied the HLA molecules involved in the CPAg-specific IgE response. PBL from

patients were incubated with CPAg, PWM, and MAb to various specificities of HLA antigens. As demonstrated in Table IV, MAb to HLA-DR framework blocked the IgE response to CPAg in a dose-dependent manner (in donor SM, at a final concentration 10⁻²; 40 pg/ml, 10⁻³; 85 pg/ml, 10⁻⁴; 120 pg/ml), whereas the other antibodies to class I, DQ and DP, or control ascites did not inhibit the response (150 to 190 pg/ml in expt. 1 and 280 to 325 pg/ml in expt. 2).

HLA molecule involved in the T-monocytes interaction. We tested whether or not HLA-DR is involved in the CPAg-driven IgE response by studying the genetic restriction in the cooperation of monocyte and monocyte-depleted PBL to respond to CPAg, because Broder et al. (33) and Indiveri et al. (34) demonstrated that the PWM-induced polyclonal Ig production can be blocked by anti-HLA-DR antibody and a factor that induces polyclonal Ig production is absorbed in anti-HLA-DR column. These findings raise the possibility that the effect of anti-HLA-DR antibody did not directly indicate the involvement of HLA-DR antigen in the antigen-driven CPAg-specific IgE response. To pinpoint the role of HLA-DR molecule, monocyte-depleted PBL from a responder SM and RO were incubated with CPAg and PWM in the presence of autologous or allogeneic monocytes. This experiment was done, based on the finding that there is no genetic restriction between the monocyte-depleted PBL and monocytes in PWM-driven nonspecific (total) IgE production, including CPAg-specific IgE Ab (data not shown). As shown in Table V, monocyte-depleted PBL revealed a trace response (16 and 8% autologous cooperation, in experiments 1 and 2, respectively). When allogeneic monocytes added to the culture shared the HLA-DR antigen, the level of specific IgE production induced was the same as in the case of autologous combination (93 to 123% in expt. 1 and 85 to 112% in expt. 2). A significantly lower IgE production was observed when they shared only class I antigen or DQ antigen instead of the DR (23 to 40% and 19 to 23%).

Suppressor T cell in nonresponders. As demonstrated in Table VI, a nonresponder JS showed a significant IgE response to CPAg when Leu-2⁺3⁺ T cells were depleted (B + Mo + Leu-3, 200 pg/ml). The autologous Leu-2⁺3⁺ T

TABLE IV
Effect of monoclonal antibodies on specific IgE response

Cell Fraction	MAb ^a	Specificity	Concentration ^b	Anti-CPAg IgE (pg/ml)
Expt. 1 PBL ^c (SM)	PA2-6	Class I	10 ⁻³	185
	B7/21	DP	10 ⁻³	190
	HU-11	DQw1	10 ⁻³	150
	HU-4	DR framework	10 ⁻²	40
			10 ⁻³	85
			10 ⁻⁴	120
	P3X	Control	10 ⁻²	180
			10 ⁻³	175
			10 ⁻⁴	155
Expt. 2 PBL ^d (RO)	PA2-6	Class I	10 ⁻³	290
	B7/21	DP	10 ⁻³	310
	HU-11	DQw1	10 ⁻³	280
	HU-4	DR framework	10 ⁻³	105
	P3X	Control	10 ⁻³	325

^a MAb of ascites form were added to culture medium throughout the culture period.

^b Final concentration of ascites in culture medium.

^c PBL donor SM: HLA-A24/-, Bw52/w60, Cw3/-, DR2/w6, Dw12/-, DQw1/w1.

^d PBL donor RO: HLA-Aw33/-, B44/-, Cw4/-, DR8/w13, DQw1/-.

TABLE V
T-monocyte interaction in IgE response to CPAg is restricted by HLA-DR^a

Monocyte Donor	HLA-A	-B	-C	-DR	-DQ	Percent Autologous Cooperation
Expt. 1: SM Autologous (-)	24, -	w52, w60	w3, -	2, w6	w1, w1	100 (190 pg/ml)
KM	24, 30	w52, -	w3, -	2, -	w1, -	16
IN	24, 31	7, w61	-	4, w6	w1, w3	93
TS	24, 26	w35, -	w3, -	4, w8	w3, -	123
HN	1, 24	40, -	w1, w3	4, 5	w3, -	40
NO	11, -	w35, w62	w3, -	4, 8	w1, w3	26
Expt. 2: RO Autologous (-)	w33, -	44, -	w4, -	8, w13	w1, -	23
YU	24, 11	w54, -	w1, -	4, 8	w1, -	100 (130 pg/ml)
SS	w33, w33	44, 44	-	w13, w13	w1, w1	8
TM	2, 24	-	w7, -	1, 2	w1, -	112
KT	2, 11	51, -	-	4, -	w3, -	85
						19
						23

^a Monocyte-depleted PBL from responders SM and RO (1.5×10^6 /ml) were co-cultured with autologous or allogeneic monocytes (0.15×10^6 /ml), CPAg ($10 \mu\text{g}/\text{ml}$ for SM and $3 \mu\text{g}/\text{ml}$ for RO) and PWM ($1 \mu\text{g}/\text{ml}$). % Autologous cooperation = $100 \times (\text{Specific IgE antibody in allogeneic combination} / \text{Specific IgE antibody in autologous combination})$. Specific IgE antibodies in culture without CPAG were 30 to 50 pg/ml in all combinations.

TABLE VI
Leu-2⁺3⁻ suppressor T cell in nonresponders

Donor ^a	Cell Fraction ^b			Anti-CPAg IgE (pg/ml)			Anti-MTAG IgE (pg/ml)		
	B + Mo	Leu-3	Leu-2	PWM only	CPAg + PWM	ΔIgE	PWM only	MTAG + PWM	ΔIgE
JS	0.5	—	—	*	*	0	Not tested	130	-130
	—	0.5	0.5	*	*	0			
	0.5	—	0.5	*	*	0			
	0.5	0.5	—	*	200	200			
MM	0.5	0.5	0.5	*	*	0	370	300	230
	0.5	0.5	—	45	170	125			
	0.5	0.5	0.5	40	50	10			
	0.5	0.5	0.25	40	70	30			
GY	0.5	0.5	—	30	120	90	165	140	75
	0.5	0.5	0.25	35	50	15			
HY	0.5	0.5	—	75	470	395	380	300	310
	0.5	0.5	0.25	35	30	-5			

^a Donor JS is not allergic either to CPAG or MTAG, whereas MM, GY, and HY are allergic to MTAG but not to CPAG.

^b Each of the cell fractions were cultured in 2 ml of culture medium per tube at an indicated concentration ($\times 10^{-6}$ /ml). Panning was performed by using anti-Leu-2a MAb in MM and anti-Leu-3a in others. *, <30 pg/ml.

cells abolished the response. A donor MM, who is allergic to MTAG but not to CPAG, revealed a significant IgE response not only to MTAG but also to CPAG when B + monocyte + Leu-2⁺3⁻ T cells were co-cultured with antigen plus PWM. The response was abolished by the autologous Leu-2⁺3⁻ T cells in a CPAG-specific manner. The same phenomena were observed in a case of donor GY and HK who are also allergic to MTAG but not to CPAG. The experiments were repeated twice, and the same results were obtained. These data indicate that nonresponders have monocytes, B cells, and Leu-2⁺3⁻ T cells that are potent for CPAG-specific IgE response, but the response is abolished by CPAG-specific suppressor T cells. However, it remains to be elucidated whether suppressor T cells were already generated in vivo or were induced in vitro, and whether the antigen specificity is in its induction or effector phase.

DISCUSSION

The important findings in this study are de novo anti-CPAG IgE antibody can be induced in vitro and the response is B cell-, monocyte-, and Leu-2⁺3⁻ T cell-depend-

ent; the response is blocked by anti-HLA-DR framework MAb; T-monocyte interaction is restricted by HLA-DR antigen; nonresponders have Leu-2⁺3⁻ suppressor T cells that suppress IgE response to CPAG, and even nonresponders will respond to CPAG when Leu-2⁺3⁻ T cells are removed. Thus we concluded that the resistance to CP, which was proved to be HLA-linked in our previous study (2), is controlled by the immune-suppression (*Is*) gene for CPAG (*Is*-CPAG) through suppressor T cells. This is the second report of *Is*-gene in the human immune response to natural antigens based on segregation and linkage analyses, as well as an analysis of the cellular mechanism of nonresponse.

Recent efforts to elucidate the regulation of IgE response demonstrated the gene controlling the IgE-specific immune response in rats (35, 36). However, difficulties in the induction of IgE response in vitro and in the measurement of the small amount of IgE have inhibited the analysis of human IgE response in vitro. Many conflicting results have been obtained in terms of "IgE induction." Some investigators claimed that PWM markedly enhances total IgE, as well as specific IgE antibody by inducing de novo synthesis in vitro (9, 14–18, 20, 23), whereas others could not confirm these findings (7–13, 21, 22). The antigen-driven IgE synthesis has also been reported by Geha et al. and Nonaka et al. (19, 20). The controversy might be well explained by the quality of anti-IgE antibody used in assays. Spiegelberg et al. (24) found that anti-IgE antibody purified by the same IgE as used in immunization possesses anti-idiotypic reactivities; thus "pseudo-IgE" can be measured, particularly after PWM stimulation. However, there is a contradictory report that such an anti-IgE antibody may react only with a Fc fragment of IgE (25). We used IgE-PS for immunization and IgE-ND for purification to rule out this possibility.

Ever since Benacerraf and McDevitt described the major histocompatibility complex-linked immune response genes (*Ir*-genes), efforts have been made to clarify the functions and structures of *Ir*-genes. The products of *Ir*-genes in mice seem to be the I-A molecule or I-E molecule determined in studies by using bml2 (37) and transgenic mice (38). These products cause mixed leukocyte reactions and act as restriction molecules in T-monocyte interaction (39), and MAb to the *Ir*-gene product abol-

ishes the antigen-specific immune response. Our results shown in Tables IV and V indicate that the function of HLA-DR antigen in the IgE response to CPag is similar to that of I-A or I-E molecules in mice, suggesting that the HLA-DR is most likely to be a product of the human *Ir*-gene to CPag. If the HLA-linked nonresponsiveness to CPag is due to deletion of the function of the *Ir*-gene, the nonresponsiveness to CPag must be an HLA-linked single recessive trait. However, our data obtained in the genetic analysis of 165 multiplex families revealed the exact opposite (1, 2):⁵ the nonresponsiveness to CPag is an HLA-linked single dominant trait. Furthermore, the data in Table VI show the existence of suppressor T cells in nonresponders, which abolish the IgE response to CPag. These findings suggest that the nonresponsiveness to CPag is controlled by the HLA-linked *Is*-gene through suppressor T cells. The observed immune response and immune suppression was clearly CPag specific as was shown in Table VI by using MTag as a control. However, at this stage, we do not know how many epitopes are on the 41,000 and 46,000 dalton molecules and which is the major one. This problem will have to be given additional attention by using inhibition tests with Mab to CPag.

Where then is the "HLA-linked *Is*-CPag" mapped? Interestingly enough, the responsiveness to CPag is associated primarily with the HLA-DQ allele (DQw3), not with HLA-DR specificities (1). This was also the case in the nonresponsiveness to SCW antigen, in which the linkage disequilibrium of the HLA-linked *Is*-gene for SCW with a specific HLA-DQ allele (HLA-DQw1) was proved (40). Our recent study revealed that HLA-DR2-Dw2-DQw1 and HLA-DR2-Dw12-DQw1 haplotypes are completely different in immune responsiveness to SCW antigen, namely Dw2 haplotype is *Is*(+), whereas Dw12 is *Is*(-) haplotype (1, 2, 40). These two haplotypes are clearly different in their DQw1 molecules, at both the protein (27) and DNA (41) levels. Therefore the difference in immune response to SCW antigen between the two haplotypes could be due to difference in their HLA-DQ molecules. Thus HLA-linked *Is*-SCW is most likely mapped within the HLA-DQ subregion (41).⁶ In this connection, there is a report by Araneo and Yowell (42) that H-2 linked suppression to chicken egg-white lysozyme in mice is mediated by suppressor effector T cells that bear the I-A determinant, which is structurally homologous to human HLA-DQ. Moreover, recent results of Ishizaka et al. (43, 44) demonstrated that the glycosylation inhibiting factor, which controls the formation of IgE suppressive factor, has a determinant of I-J. From all of these observations, it is conceivable that the *Is*-CPag is mapped in the HLA-D region, most likely within the HLA-DQ subregion. It remains to be determined how suppressor T cells mediate the nonresponsiveness to CPag; where the HLA-linked *Is*-CPag is precisely mapped; and what the nature of the gene product of *Is*-CPag is.

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