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DRUG-SPECIFIC IMMUNE RESPONSES INDUCED BY IMMUNIZATION WITH DRUGS IN GUINEA PIGS AND MICE

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Abstract.....In order to develop a system for evaluating the allergenicity of drugs in clinical use, we tested drugs for the ability to induce drug-specific immune responses in guinea pigs and mice. Test drugs were benzylpenicillin, procainamide, hydralazine, isoniazid, α -methyldopa, D-penicillamine, captopril, sulfamethoxazole and 2,4-dinitrochlorobenzene (DNCB), which are known to induce allergic responses in man including hypersensitivity reactions and drug-induced auto-immune responses. Guinea pigs were immunized with an emulsion of complete Freund's adjuvant (CFA) and 25 mg of each drug. Mice were immunized with an emulsion of CFA and 2 mg of each drug or a mixture of aluminum hydroxide gel and 2 mg of each drug. In order to examine drug-specific immune responses, we employed detection of antibodies by enzyme-linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis tests, active systemic anaphylaxis (ASA) tests and delayed type hypersensitivity (DTH) tests. In guinea pigs, drug-specific antibodies were detected following immunization with benzylpenicillin, procainamide, hydralazine, isoniazid, captopril, sulfamethoxazole or DNCB. Some of these drugs were also positive in DTH tests and/or ASA tests. In mice, however, only DNCB gave positive results. Therefore, our system involving immunization of guinea pigs with CFA emulsion of a drug and detection of drug-specific immune responses is considered to be an effective test method for evaluating drug allergenicity.

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Key words : Immunogenicity, drug allergy, hypersensitivity, drug-induced autoimmunity.

INTRODUCTION

Although drug allergic reactions are a serious problem, it has not been possible to identify them prior to marketing of drugs in most cases. Animal models are believed to be unsuitable to predict this adverse effect. A few test systems including popliteal lymph node assay in mice have been proposed (Gleichmann et al., 1984, 1989 ; Kammüller et al., 1989), but none is yet routinely employed as a preclinical test. The mechanism of drug hypersensitivity is believed to involve conjugation of the drug with macro-molecules or polymerization (de Weck et al., 1968 ; Amos and Park, 1985 ; Park et al., 1987). Although penicillins were shown at the molecular level to induce allergic reactions by this mechanism (Levine and Ovary, 1961 ; Ahlstedt and Kristofferson, 1982), the mechanisms in the cases of other drugs have not been clarified. Moreover, identification of the causative drug in hypersensitivity reactions in the clinical setting is not necessarily easy. On the other hand, many drugs including procainamide, isoniazid, α -methyldopa and hydralazine have been identified as agents causing auto-immune responses (Stratton, 1985 ; Totoritis and Rubin, 1985 ; Bigazzi, 1988 ; Adams and Hess, 1991). D-Penicillamine and captopril are also considered to have potential for causing auto-immune reactions (Totoritis and Rubin, 1985 ; Adams and Hess, 1991). In the previous report, we showed that procainamide, isoniazid, hydralazine and benzylpenicillin caused drug-specific immune responses such as antibody production and delayed-type hypersensitivity (DTH) in guinea pigs, and suggested that tests for drug-specific immune responses might provide a possible test system for predicting drug allergic reactions (Katsutani and Shionoya, 1992). To test the feasibility of this approach, further studies including tests on other drugs in guinea pigs and tests in mice have been performed.

MATERIALS AND METHODS

Animals :

Female Hartley guinea pigs, 4 weeks of age, male BALB/c mice, 3 weeks of age, and male Sprague-Dawley rats, 7 weeks of age, were purchased from Japan SLC Co. (Shizuoka, Japan). They were acclimated for 2 weeks before use.

Agents :

Procainamide hydrochloride, isoniazid, D-penicillamine, hydralazine hydrochloride, sulfamethoxazole, bovine serum albumin (BSA), ovalbumin (OVA), Evans blue, γ -maleimidobutyric acid N-hydroxysuccinimide ester (GMBS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) were purchased from Sigma Chemical Co. (St. Louis, MO). Benzylpenicillin potassium salt was

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purchased from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). Sodium nitrite, 2,4-dinitrochlorobenzene (DNCB), glutaraldehyde, aluminum chloride and sodium hydroxide were purchased from Katayama Chemical Co. (Osaka, Japan). DL- α -Methyldopa was purchased from Nakarai Tesque Inc. (Kyoto, Japan). 2,4-Dinitrobenzenesulfonic acid sodium salt (DNBS) was purchased from Tokyo Kasei Co. (Tokyo, Japan). Complete Freund's adjuvant (CFA) was purchased from Difco Laboratories (Detroit, MI). Aluminum hydroxide gel was prepared by mixing equal volumes of 0.1 M AlCl_3 and 0.3 M NaOH.

Preparation of drug-protein conjugates :

Each drug was conjugated with proteins. The concentration of the conjugates was determined by means of the BCA Protein Assay Reagent (Pierce, Rockford, IL). The amounts of drug bound to BSA or OVA were estimated by ultraviolet or visible (in the case of dinitrophenyl-protein conjugates) absorption analysis, and the results are presented in Table 1.

1) procainamide-protein conjugates

Procainamide (0.4 g) was dissolved in 5 ml of 0.5 N HCl and the solution was mixed with 10 ml of 2% sodium nitrite. The admixture was kept for 1 hour at 4°C, then was neutralized by adding 1 N NaOH. The reactant was divided into two equal portions, which were mixed with 5 ml of 4% BSA or OVA, respectively, in 0.1 M borate buffer, pH 8.5. The admixture was kept overnight at 4°C and then dialyzed against phosphate-buffered saline (PBS).

2) isoniazid-protein conjugates and hydralazine-protein conjugates

Aqueous isoniazid or hydralazine solution (0.2 g), aqueous BSA or OVA solution (0.2 g) and aqueous EDC solution (0.6 g for isoniazid or 0.4 g for hydralazine) were mixed and kept overnight at room temperature. The reactants were dialyzed against PBS.

3) penicillin-protein conjugates

Benzylpenicillin (1.5 g) and BSA or OVA (1.0 g) were dissolved in distilled water. The mixture was stirred for 1 hour, during which time the pH was maintained at 11.5 by adding 1 N NaOH. The reactant was dialyzed against PBS.

4) methyldopa-protein conjugates and penicillamine-protein conjugates

One of the drugs (50 mg) and BSA or OVA (0.2 g) were dissolved in 2 ml of distilled water. An equimolar amount of 2% glutaraldehyde solution was added, and the mixture was held for 2 hours at room temperature. The reactants were dialyzed against PBS.

5) captopril-protein conjugate

GMBS (25 mg) was dissolved in tetrahydrofuran and added to a mixture of aqueous captopril solution (0.2 g) and aqueous BSA or OVA solution (0.2 g), and kept for 2 hours at room temperature. The reactants were dialyzed against PBS.

6) 2,4-dinitrophenyl (DNP)-protein conjugate

DNBS (0.5 g) and BSA or OVA (0.5 g) were dissolved in 2.5% aqueous sodium carbonate solution and kept for 3 hours at room temperature. The reactants were

Table 1. Number of haptens of drug-protein conjugates⁺.

Drug	Number of haptens [‡]	
	BSA conjugate	OVA conjugate
Procainamide	44.3	19.9
Isoniazid	46.0	27.9
Hydralazine	60.4	35.2
Sulfamethoxazole	13.0	9.8
D-Penicillamine	NT*	NT
α -Methyldopa	28.8	16.7
Captopril	NT	NT
Benzylpenicillin	38.7	20.9
DNBS	20.0	7.2

+ A drug was bound to BSA or OVA as described in Materials and Methods. The amount of drug bound to BSA or OVA was estimated by means of ultraviolet or visible (in the cases of DNP-protein conjugates) absorption analysis.

[‡] Each figure represents the number of haptens bound to one protein molecule.

* Not tested because these compounds have neither ultraviolet nor visible absorption, or the absorption is masked by strong ultraviolet absorption of the proteins.

dialyzed against PBS.

Immunization and blood collections :

Immunization procedures for guinea pigs and mice are summarized in Table 2. For immunization of guinea pigs, 25 mg of one of the drugs or 0.25 mg of one of the drug-OVA conjugates was dissolved or suspended in a physiological saline solution and emulsified with an equal volume of CFA. Insoluble drugs were suspended in CFA and emulsified with a physiological saline solution. The emulsion was subcutaneously (sc) injected at a total volume of 0.5 ml (0.125 ml per site in four separate positions) into the back. Immunization was repeated two weeks later.

For immunization of mice, 2 mg of one of the drugs or 0.1 mg of one of the

Table 2. Immunization of guinea pigs and mice.

Animal species	Test compound	Dose (mg/animal)	Adjuvant	No. of injections	No. of animals
Guinea pig	Drug	25	CFA ⁺	2	10
	Drug-OVA	0.25	CFA	2	2 or 5
Mouse	Drug	2	CFA	2	5
	Drug	2	Alum [‡]	2	5
	Drug-OVA	0.1	CFA	2	2
	Drug-OVA	0.1	Alum	2	2

+ Complete Freund's adjuvant

[‡] Aluminum hydroxide gel

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drug-OVA conjugates was dissolved or suspended in a physiological saline solution. This solution was mixed with an equal volume of aluminum hydroxide gel (20 mg gel/ml) or emulsified with an equal volume of CFA. The mixture or the emulsion was injected intraperitoneally (ip) at a volume of 0.2 ml. Immunization was repeated two weeks later.

Blood samples were collected from the orbital plexus 2 weeks after the second injection. Ten guinea pigs or five mice were used for immunization with each drug and 2 or 5 animals were used for immunization with each drug-OVA conjugate.

Enzyme-linked immunosorbent assay (ELISA) :

ELISA techniques as described elsewhere (Katsutani and Shionoya, 1992) were employed in order to detect drug-specific antibodies. A serum sample was considered to be positive when the absorbance of the serum tested was greater than the mean plus 3 standard deviations of all 6 serum samples of the negative control group. The antibody titer was determined as the reciprocal of the maximum positive dilution.

Inhibition of ELISA was evaluated by the assay of a sample which had been incubated with the drug solution (5 mg/ml) for 1 hour at 37°C. As a control, a physiological saline solution was used instead of the drug solution. The lack of any inhibition reaction by the drug solutions used was confirmed by tests using antiserum to OVA.

DTH tests :

Under pentobarbital anesthesia, guinea pigs immunized with a drug had their abdomens shaved with an electric clipper. An aqueous drug solution or an aqueous drug-BSA conjugate solution at a volume of 0.1 ml was intradermally (id) injected into the shaved area. DTH reactions, erythema and/or induration at the injected sites were observed 24 and 48 hours after the id injections. The absence of any reaction induced by id injections of a drug solution or a drug-BSA conjugate solution alone was confirmed by control tests using untreated animals.

Passive cutaneous anaphylaxis (PCA) tests :

Under pentobarbital anesthesia, rats had their back shaved with an electric clipper. Serum samples from mice were id injected into the shaved back. Twenty-four hours after the injections, the corresponding drug-BSA conjugate (1 mg/animal), which was dissolved in a physiological saline solution or 7 M urea solution and then mixed with an equal volume of 2% Evans blue dye solution, was intravenously (iv) injected into the tail vein. The animals were killed 30 minutes later. The skin of the back was removed and the reactions (blue-colored spots) at the sites of id injections were examined.

Active systemic anaphylaxis (ASA) tests :

Guinea pigs immunized with a drug or a drug-OVA conjugate were challenged by injecting the corresponding drug-BSA conjugate (1 mg/animal), which was dissolved in a physiological saline solution or 7 M urea solution, into the matatarsus vein, and the animals were examined for systemic anaphylactic symptoms (such as

nose rubbing, pilo-erection, weakness, dyspnea, coughing, sneezing, vomiting, wheezing, convulsions, collapse and death) for 30 minutes after the injection.

RESULTS

Guinea pig studies :

Drug-specific antibodies were detected by means of ELISA and the results are presented in Fig. 1. Antibodies to the immunized drug were produced with high titers by immunization with benzylpenicillin, hydralazine, isoniazid or DNCB.

Fig. 1. Drug-specific antibody responses induced by immunization with drugs or drug-OVA conjugates in guinea pigs. A drug (25 mg/animal) or a drug-OVA conjugate (0.25 mg/animal) was injected together with CFA, and a blood sample was tested for antibodies to the corresponding drug by ELISA. Antibody titer from the positive animals and positive rate (beside each bar) are presented.

Antibodies were detected with rather low titers in the sera of the animals immunized with procainamide, sulfamethoxazole or captopril, though some animals treated with these drugs did not respond. Immunization with all drug-OVA conjugates induced drug-specific antibody responses, as might be expected. Specificity of the produced antibodies for the immunized drug was evaluated by ELISA inhibition. The serum samples were incubated with the corresponding drug solution or saline solution as a control, and tested for antibodies to the corresponding drug. The results, expressed as % inhibition, are presented in Table 3. Inhibition reactions were seen with all antibodies tested and specificity for the immunized drug was determined.

Results of DTH reactions of the animals immunized with drugs are presented in Table 4. DTH reactions were seen in the animals immunized with procainamide,

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Table 3. ELISA inhibition of the antibodies in serum samples of guinea pigs immunized with drugs +.

Immunized with	% inhibition (mean $\pm$ S. D.)
Procainamide	80.3 $\pm$ 14.6
Isoniazid	37.4 $\pm$ 13.1
Hydralazine	58.8 $\pm$ 13.2
Sulfamethoxazole	86.5 $\pm$ 17.7
Captopril	51.3 $\pm$ 7.0
Benzylpenicillin	32.8 $\pm$ 12.3

+ Inhibition of ELISA was evaluated by assay of a sample which had been incubated with the corresponding drug (5 mg/ml) for 1 hour at 37° C. As a control, a physiological saline solution was used instead of the drug solution. The result was expressed as % inhibition with respect to the control. Each value was represented as mean $\pm$ S. D. from the samples which showed ELISA positive.

isoniazid or hydralazine. While reactions of the animals immunized with procainamide were provoked by id injections of both the drug solution and the drug-BSA conjugate, reactions of the animals immunized with isoniazid or hydralazine were only provoked by id injections of either the drug solution or the drug-BSA conjugate. DNCB, which is a well-known contact sensitizer, was not tested because of its poor solubility in water.

ASA reactions were tested by an iv injection of the drug-BSA conjugate in animals immunized with drugs or drug-OVA conjugates, and the results are presented in Table 5. The animals immunized with benzylpenicillin, isoniazid or DNCB exhibited anaphylactic reaction. The animals immunized with all the drug-OVA conjugates except for hydralazine-OVA conjugate also exhibited anaphylactic reaction. Hydralazine-BSA conjugate was poorly soluble in water, and this was considered to account for the lack of reaction in the animals immunized with the OVA conjugate, because these animals had abundant serum antibodies to the drug, which were detectable by ELISA.

Mouse studies :

The drug-specific antibodies were examined by ELISA and PCA tests, and the results are presented in Fig. 2. In ELISA, the antibodies were assayed by using two kinds of enzyme-linked antibodies, i. e., the antibodies specific for whole molecules of IgG which are assumed to react with all kinds of antibodies, and antibodies specific for IgE. Antibodies to DNCB were detected, but no antibodies were detected in the serum samples of the animals immunized with the other drugs in both systems. The antibodies to DNCB were only induced by immunization with CFA emulsion of the drug. Antibodies were detected in the serum samples of the animals immunized with all the drug-OVA conjugates in the detection system for IgG. On the other hand, antibodies were detected only in the serum samples of the animals

Table 4. DTH reactions induced by immunization with drugs in guinea pigs⁺.

Immunized with	Observation after id injection(hr)	DTH reaction†					Drug-BSA conjugate(μ g/site)				
		0.1	1	10	100	0.01	0.1	1	10		
Procainamide	24	0/5	0/5	2/5(6.0)	5/5(7.3)	0/5	1/5(5.0)	5/5(4.5)	NT*		
	48	0/5	0/5	1/5(4.6)	5/5(6.9)	0/5	1/5(4.8)	5/5(5.0)	NT		
Isoniazid	24	0/5	0/5	0/5	0/5	0/5	0/5	2/5(4.8)	4/5(5.2)		
	48	0/5	0/5	0/5	0/5	0/5	0/5	1/5(4.1)	4/5(3.9)		
Hydralazine	24	0/5	1/5(6.5)	2/5(5.7)	4/5(6.0)	0/5	0/5	0/5	NT		
	48	0/5	0/5	2/5(4.1)	3/5(4.2)	0/5	0/5	0/5	NT		
Sulfamethoxazole	24	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
	48	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
D-Penicillamine	24	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
	48	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
α -Methyldopa	24	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
	48	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
Captopril	24	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
	48	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
Benzylpenicillin	24	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		
	48	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5		

+ DTH reactions were induced by id injections of the corresponding drug or the corresponding drug-BSA conjugate.

† DTH reactions, erythema and/or induration at the injected sites were observed 24 and 48 hours after the id injections.

Positive rate and mean diameter of the reactions from the positive animals in the parenthesis are presented.

* Not tested because of poor solubility in water.

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Table 5. ASA reactions induced by immunization with drugs in guinea pigs⁺.

Immunized with	No. of animals tested	No. of animals responding				
		ASA grading [‡]				
		—	±	+	++	+++
Procainamide	5	5	0	0	0	0
Isoniazid	5	0	0	0	0	5
Hydralazine	5	5	0	0	0	0
Sulfamethoxazole	5	5	0	0	0	0
D-Penicillamine	5	5	0	0	0	0
α-Methyldopa	5	5	0	0	0	0
Captopril	5	5	0	0	0	0
Benzylpenicillin	5	0	0	0	0	5
DNCB	5	0	0	0	0	5
Procainamide-OVA	2	0	0	0	0	2
Isoniazid-OVA	2	0	0	0	0	2
Hydralazine-OVA	2	2	0	0	0	0
Sulfamethoxazole-OVA	2	0	0	0	0	2
Penicillamine-OVA	2	0	0	0	0	2
Methyldopa-OVA	2	0	0	0	0	2
Captopril-OVA	2	0	0	0	0	2
Penicillin-OVA	2	0	0	0	0	2
DNP-OVA	2	0	0	0	0	2

+ Guinea pigs immunized with a drug or a drug-OVA conjugate were iv injected with the corresponding drug-BSA conjugate (1 mg/animal). Systemic anaphylactic reactions 30 minutes after the injection were examined.

‡ The reactions were graded as follows: nose rubbing and/or pilo-erection, ±; weakness, dyspnea, coughing, sneezing, vomiting and/or wheezing, +; convulsions and/or collapse, ++; death, +++.

immunized with DNP-OVA conjugate in the detection system for IgE. PCA tests gave positive results in the animals immunized with DNCB accompanied by CFA, and with some drug-OVA conjugates.

DISCUSSION

We have previously shown that procainamide, hydralazine, isoniazid and benzylpenicillin are immunogenic in guinea pigs, and suggested that binding with proteins is the initial step in the induction of immunological side effects including hypersensitivity reactions and drug-induced auto-immune responses (Katsutani and Shionoya, 1992). The induction of drug-hypersensitivity reactions is considered to involve conjugation of drugs with macro-molecules (Amos and Park, 1985 ; Park et al., 1987), but little is known about the mechanism of drug-induced auto-immune responses. However, several possibilities involving conjugation with macro-

Fig. 2. Drug-specific antibody responses induced by immunization with drugs or drug-OVA conjugates in mice. A drug (2 mg/animal) or a drug-OVA conjugate (0.1 mg/animal) was injected together with CFA (■) or aluminum hydroxide gel (□), and a blood sample was tested for antibodies to the corresponding drug by ELISA and PCA tests. In ELISA, two kinds of enzyme-linked antibodies were used, i. e., antibodies to mouse IgG (heavy and light chains specific) and antibodies to mouse IgE (Fc specific).

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molecular proteins, including major histocompatibility complex (MHC) molecules, have been proposed (Class and van Rood, 1985 ; Goldman et al., 1991). Moreover, the use of CFA is considered to be effective to induce reactions based on drug metabolism, because CFA can activate leukocytes including macrophages and polymorphonuclear leukocytes, and some drugs are effectively metabolized by the activated leukocytes, which have MHC class II molecules on their cell surface (Uetrecht et al., 1988 ; Uetrecht, 1989). In order to construct test systems which can be used to predict immunotoxicity of drugs in clinical use, more drugs which are clinically known to induce allergic effects, and other possible test systems than guinea pigs must be investigated. In this work we have examined the immunogenic properties of several drugs which have been shown to be immunogenic and some other drugs in guinea pigs and mice. The dose employed in the mouse studies was similar to that in the guinea pig studies per body weight of the animal, i. e. 25 mg/animal for guinea pigs and 2 mg/animal for mice. However, in order to inject the same volume of the adjuvants, the same amounts of drugs were administered without taking into account minor variations of body weights of the animals tested. The routes of administration were selected as sc for guinea pigs and ip for mice because these routes are efficient for immunization compared with other routes such as oral and iv routes (Kabat and Mayer, 1961). Antibody responses with high antibody titers following immunization with drug-OVA conjugates by the same routes as the immunization with drugs showed that the procedures used were efficient for immunization. In addition to CFA, aluminum hydroxide gel was used as an adjuvant in the mouse studies because aluminum hydroxide gel is considered to be suitable to induce homocytotropic antibody responses (Kabat and Mayer, 1961). All the results are summarized in Table 6. In guinea pigs, most drugs were immunogenic and the ELISA test could detect weak immunogenic reactions.

Table 6. Summary of the immune responses induced by drugs in guinea pigs and mice⁺.

Immunized with	Guinea pig studies			Mouse studies	
	ELISA	DTH	ASA	ELISA	PCA
Procainamide	+	+++	—	—	—
Isoniazid	+++	++	+++	—	—
Hydralazine	+++	++	—	—	—
Sulfamethoxazole	+	—	—	—	—
D-Penicillamine	—	—	—	—	—
α -Methyldopa	—	—	—	—	—
Captopril	+	—	—	NT [‡]	NT
Benzylpenicillin	+++	—	+++	—	—
DNCB	+++	NT	+++	+	+

+ Each test was graded according to intensity of reactions

‡ Not tested

However, most of those which were immunogenic in guinea pigs could not induce drug-specific immune responses in mice. This does not mean that mice are immunologically less susceptible, since mice responded adequately to DNCB and DNP-BSA conjugate. Besides, mice are known to respond sensitively to protein antigens such as OVA. In order to conclude that mouse tests are really useless for evaluation of drug allergenicity, further tests using other strains than BALB/c mice are needed. ELISA is considered to be a more effective method for detection of drug-specific immune responses than traditional biological methods such as DTH, ASA and PCA reactions, and our studies demonstrated that the highest positive rate was obtained by ELISA, as shown in Table 6.

DTH reactions to procainamide were induced by both procainamide and its conjugate. DTH reactions to isoniazid were induced by the drug-protein conjugate, while the reactions to hydralazine were induced by the drug alone. Sensitization and elicitation of the DTH reaction require antigenic determinants which are associated with MHC class II molecules. For antigen presentation by antigen-presenting cells (APC), two pathways can be considered. One way involves antigen presentation through uptake, processing and association with MHC class II molecules on the surface of APC, such as with most protein antigens. The other involves direct covalent binding or association with the antigenic determinant on the cell surface of APC, such as with dinitrofluorobenzene (Maggs et al., 1986) or with superantigen peptides (Dellabona et al., 1990 ; Gascoigne and Ames, 1991). The results of the present study revealed that injection of both a drug alone and drug-protein conjugate are required for the detection of DTH reactions, because we cannot predict which one will be effective to induce DTH reactions.

It has been considered that only mouse IgE can bind with rat mast cells and cause PCA reactions (Mota and Wong, 1969 ; Braga and Mota, 1976). However, our results indicated that the ELISA system specific for mouse IgE could not detect the antibodies which were detected by rat PCA tests. This showed that rat PCA tests could detect not only mouse IgE but also mouse IgG. While rat PCA reactions failed to be induced by mouse IgG in most cases, this may be because of low affinity of mouse IgG for rat mast cells, as suggested by König et al. (König et al., 1974). Moreover, the studies which found that mouse IgG failed to induce rat PCA reactions were carried out by using OVA as an antigen, while our results were obtained by using anti-hapten antibodies and the hapten-protein conjugate as an eliciting antigen. The density of the antigenic determinant on a hapten-carrier antigen and a protein antigen is different, and this might affect the result. Our result seems to be the first showing that rat PCA reactions can be induced by mouse IgG. However, further investigation is needed to confirm this.

Kammüller et al. (1989) presented the results of popliteal lymph node reactions in mice by many drugs, which might induce auto-immune responses in man, and proposed this as a possible test system for prediction of immunotoxicity. However, although some drugs were positive, others, which were expected to become toxic

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after being metabolized, were negative in the assay system. Other systems have been proposed for identifying contact sensitizers, including the well-known guinea pig maximization test (Magnusson and Kligman, 1969 ; Shlede et al., 1989). All these systems are potentially available for detecting drugs which can cause immunological adverse effects. Nevertheless, our system, which involves detection of drug-specific antibodies in guinea pigs, has the advantage of excluding non-specific reactions. In other test system non-specific reactions, such as erythema or proliferation of draining lymph nodes, are induced by non-immunological mechanisms, while specific antibodies can not be produced by other mechanisms. However, information about drug metabolism is needed in order to detect drug-specific antibodies effectively. For example, arylamines including procainamide and sulfamethoxazole are known to form active metabolites by reaction at the amino substituent of the phenyl group (Uetrecht, 1985 ; Rieder et al., 1988). We produced the drug-protein conjugates by the reaction of the diazotized compounds with proteins, and these conjugates effectively detected antibodies produced. Although immunological cross-reactions may provide positive results, drug-protein conjugates should be produced based on information about drug metabolism, because otherwise a negative result may not imply the absence of drug-specific antibodies. For example, D-penicillamine was negative in these studies. This conjugate was produced by bridging between the amino groups of the drug and the protein, though it has been proposed that the thiol group of the drug is actually involved in conjugation with proteins (Coleman et al., 1988). Therefore, there is still a possibility that a positive result would be obtained with a different conjugate.

We have demonstrated that drugs can induce drug-specific immune responses in guinea pigs when CFA is used as an adjuvant, though these drugs did not induce such responses in mice. These drugs are known to cause allergic adverse effects including hypersensitivity and auto-immune responses in clinical use. These facts suggest that the immunogenic potential is related to not only hypersensitivity reactions but also drug-induced auto-immune responses, and that leukocytes activated by CFA play important roles in enhancing immunogenic potential, including drug metabolism and antigen presentation. Activated leukocytes also seem to play important roles in inducing auto-immune reactions as clinical adverse effects (Uetrecht, 1989). Thus, we consider that our test system, based on immunization of guinea pigs with drugs and CFA, and detection of drug-specific immune responses by ELISA, has the potential to become a useful test system for preclinical evaluation.

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