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IMMUNOLOGIC BASIS FOR ADVERSE REACTIONS TO RADIOGRAPHIC CONTRAST MEDIA

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Abstract

The lymphocyte transformation test (LTT) was used to elucidate whether certain side effects induced by radiographic contrast media have an immunologic etiology. Groups studied were: 8 patients who had previously experienced adverse reactions in association with urography, 6 patients who underwent urography without notable side reactions, 17 occupationally exposed nurses, and 9 unexposed controls. The lymphocytes from 2 hypersensitive patients and from 11 nurses exhibited a positive proliferative response to amidotrizoate. Five nurses who had shown a positive response, had a previous history of hypersensitivity reactions when handling contrast media, whereas the remaining 6 were free of symptoms. Amidotrizoate-specific memory cells were absent in patients who underwent urography without signs of hypersensitivity and in 7/9 of unexposed control subjects. Lymphocytes from patients sensitive to amidotrizoate cross-reacted to structurally related ionic contrast media while non-ionic contrast agents did not induce proliferation of the lymphocytes. Thus, ionic radiographic contrast agents have antigenic properties in man. Irradiated mixtures of radiographic contrast media and serum proteins were, in general, not effective in inducing an LTT response.

Key words: Contrast media, adverse reactions; —, comparative studies; —, lymphocyte transformation.

Various side effects have been associated with the clinical use of radiographic contrast media (1, 2, 9, 11, 12). Clinical surveys show that the incidence of mild reactions including flushing, nausea, and headache is considerable. More severe side effects, such as asthma or anaphylactoid reactions, are rare, but the problem is sufficiently serious to be of concern (11). The introduction of "low osmolality" radiographic contrast media (non-ionic agents like iohexol, iopamidol, and ioxaglate) has considerably reduced the incidence of side effects (8). However, the significantly higher costs associated with the use of the latter category of compounds, as well as the fact that a majority of patients do not react adversely towards ionic contrast media, has resulted in a continued wide-scale use of conventional products in many clinics. The lack of reliable predictive tests for detecting hypersensitivity (9, 10, 12) introduces an element of uncertainty when attempting to select the most appropriate type of contrast medium for each patient.

Side effects induced by radiographic contrast media have a complex etiology, and several pathogenic mechanisms have been suggested (11). Since many of these adverse reactions resemble allergic manifestations, an immunologic mechanism has been proposed by some authors (6, 11). Iodine, linked to proteins, constitutes a powerful antigenic determinant (3, 14). In a previous publication, the in vitro and in vivo formation of iodinated serum proteins following gamma irradiation in the presence of two commonly used radiographic contrast media was demonstrated (18). On the basis of these results it was suggested that radiographic contrast agents may serve as antigens which indirectly could trigger an allergic cross reaction in certain individuals previously sensitized to structurally similar iodo-proteins. An immunologic response to an antigen implicates the presence of specific memory lymphocytes in the blood of sensitized individuals. In vitro proliferation of memory cells induced by a specific antigen constitutes the basis of the lymphocyte transformation test (LTT). This methodology has previously proved to be a useful tool for the elucidation of the etiology of hypersensitivity reactions in occupationally exposed workers employed in the pharmaceutical industry (21, 23, 24) as well as for the discrimination between irritative responses and immunologically mediated reactions to isothiazolone based bactericidal agents (22). In the present investi-

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gation, the lymphocyte responses to various radiographic contrast media have been studied in patients as well as in occupationally exposed nurses. Immunologic cross reactions to iodinated serum proteins, formed upon irradiation as well as by chemical methods in vitro, were also investigated.

Material and Methods

Patients and control subjects. The clinical characteristics of 8 patients who had experienced various hypersensitive reactions in the past following the intravenous injection of amidotrizoate are shown in Table 1. Similar data are given in Table 2 for 17 nurses employed in the radiologic clinic of Danderyds Sjukhus who had been occupationally exposed to various ionic and non-ionic contrast media for up to 30 years. In this clinic iohexol has been the only non-ionic radiographic contrast medium used. Some of the nurses experienced hypersensitivity reactions including rhinitis, conjunctivitis, and urticaria while handling these agents. Patients who had undergone urography without noticeable side reactions (6 patients) as well as laboratory personnel without previous exposure to radiographic contrast media (9 persons) were also studied.

Lymphocyte transformation test (LTT). LTT was performed as described previously (21, 24), with minor modifications. In brief, 50 to 70 ml venous blood was collected in vacutainer tubes (Venoject; Mediplast AB, Stockholm, Sweden). The blood samples were immediately transferred to sterile Erlenmeyer flasks containing glass beads and defibrinated by shaking for 10 min. A 30 percent aliquot of the total blood volume was transferred to a Ficoll-Isopaque gradient (Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged to obtain lymphocytes and monocytes (5). The remaining 70 percent of the defibrinated blood was diluted 1:1 with RPMI 1 640 medium and depleted of monocytes by treatment with carbonyl iron (GAF carbonyl iron, GAF Corp., N.Y., USA). After a 30 min incubation at 37°C with occasional stirring, the iron-phagocyting cells were retained by means of a magnet whereas the monocyte-depleted blood was separated on a Ficoll-Isopaque gradient. After separation, both cell fractions were combined and the cells washed and diluted in RMPI 1 640 medium containing 10 mmol of N′-2-hydroxyethyl-piperazine-N′-2-ethanesulfonic acid and 1 g of $NaHCO_3$ per liter. The medium was supplemented with gentamycin (Garamycina; Schering Corp., Kenilworth, NJ, USA), glutamine, and 10 per cent heat inactivated human AB^+ serum. A lymphocyte suspension (10^6 cells/ml) was incubated with a wide range of concentrations of radiographic contrast medium, or with dialyzed protein conjugates prepared as described below. The total culture volume was 2 ml. Pokeweed mitogen (PWM; Gibco, Grand Island, N.Y., USA) at a concentration of 1 µg/ml and/or PPD (purified protein derivative, Statens Seruminstitutt, Copenhagen, Denmark) at a concentration of 5 µg/ml was used as a positive control of lymphocyte proliferation (the majority of the adult population in Sweden has been vaccinated with Calmette-Guérin vaccine and exhibits a Mantoux-positive skin test). Control lymphocytes (3 parallel cultures) were incubated without any antigen. The cultures were then incubated at 37°C and 100 percent humidity in 6 percent CO_2 for 5 days. Amidotrizoate (3,5-diacetamido-2,4,6-triiodobenzoic acid; Urografin) was obtained from Schering AG (Berlin, Germany), metrizoate (3-acetamido-2,4,6-triiodo-5-(N-methylacetamido) benzoic acid, Isopaque) and iohexol (N,N′-bis(2,3-dihydroxypropyl)-5-[N-(2,3-dihydroxypropyl) acetamido]-2,4,6-triiodoisophthalic acid amide, Omnipaque) from Nycomed (Oslo, Norway), iothalamate (5-acetamido-2,4,6-triiodo-N-methylisophthalic acid amide; Conray) and iopamidol (N,N-bis[2-hydroxy-1-(hydroxymethyl) ethyl] -2,4,6- triiodo -5- lactamidoisophthalic acid amide, Iopamiro) from Astra-Meditec (Mölndal, Sweden), iopromide (N,N′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-(2-methoxyacetamido)-N-methylisophthalic acid amide, Ultravist) from Schering AG (Berlin, Germany), and human serum albumin (HSA) from Kabi AB (Stockholm, Sweden). All other chemicals used were of analytical grades.

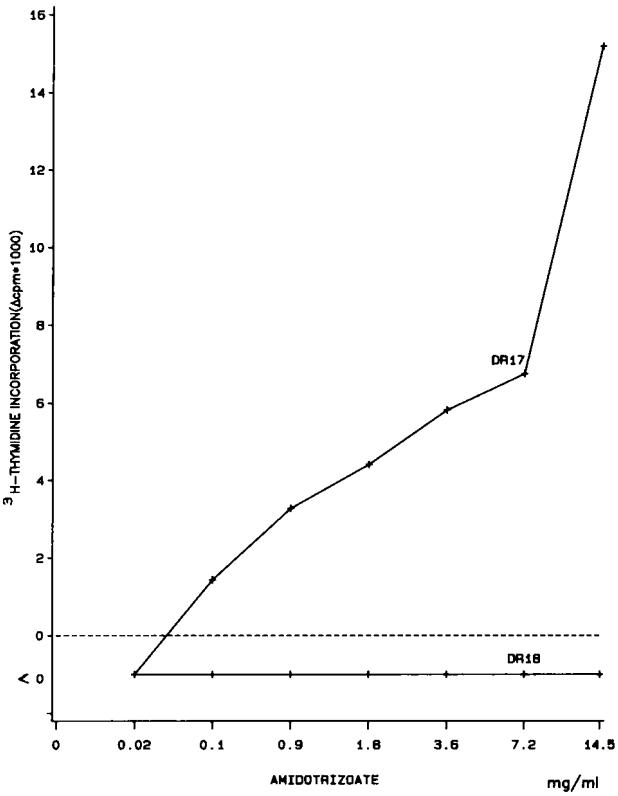


Fig. 1. Lymphocyte proliferation to amidotrizoate in patient DR17. This patient had experienced anaphylactic shock following infusion of amidotrizoate. Lymphocytes from patient DR18, who underwent urography without side effects, were tested in the same experiment.

Incorporation of radiolabeled thymidine. After 5 days, one ml of medium was removed from each culture and 0.2 ml of cell suspension were transferred as triplicate samples to V-shaped microplates (Titertek; Flow Labs, Irvine, Scot-

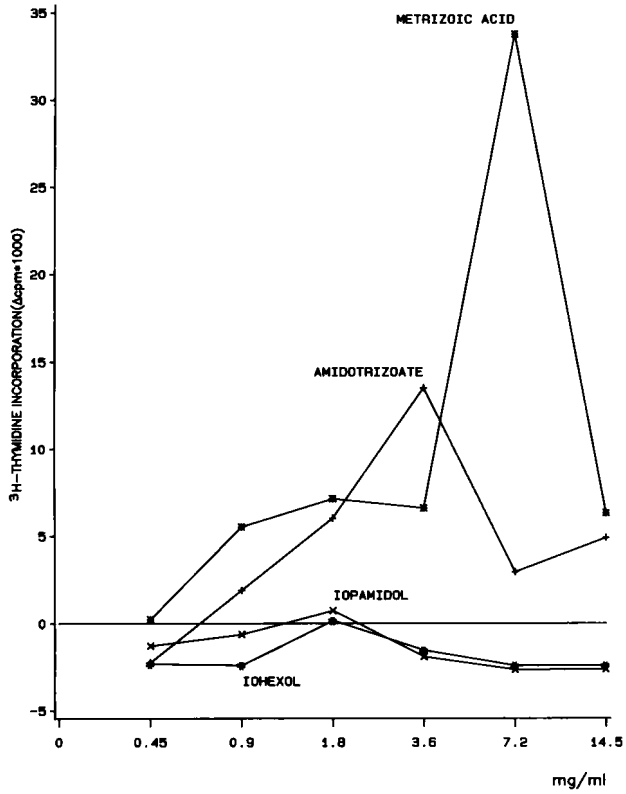


Fig. 2. Lymphocyte proliferation in occupationally exposed nurse (DR32). This individual showed hypersensitivity symptoms (rhinitis, urticaria) while working with amidotrizoate.

Table 1

Clinical data for patients with hypersensitivity reactions following urography with amidotrizoate

Code	Age	Sex	Year and no. of previous exposures	Symptoms
DR1	62	M	1986 (1)	Anaphylactic shock, asthma
DR3[a]	50	M	1982 (1)	Anaphylactic shock
DR14[b]	47	F	1978 (1)	Urticaria, Quincke's edema
DR17[c]	33	M	1978 (1)	Anaphylactic shock
DR19	51	M	1977 (2)	Vomiting, dyspnea
DR21	69	M	1974 (1)	Anaphylactic shock, urticaria
DR24[d]	44	M	1976 (1)	Urticaria, itching (eyes, hands, trunk)
DR25	44	F	1983 (10)	Urticaria, Quincke's edema, coughing

[a]Patient claims to suffer from penicillin allergy.
[b]Reacts to strawberries; atopic predisposition.
[c]Reacts to cats.
[d]Reacts to insect bites.

land) and pulsed with 37 kBq of 5′-^{3}H-thymidine (TRK 328, Radiochemical Centre, Amersham, England; specific activity 185–518 GBq/mmol) for 4 hours. The cultures were harvested in a Skatron (Skatron, Lier, Norway) semiautomatic cell harvester, and the radioactivity measured in a liquid

Table 2

Clinical data for female nurses occupationally exposed to radiographic contrast media

Code	Age	Duration of exposure (years)	Symptoms upon exposure
DR9[a]	39	14	None; underwent urography (1981) without side effects
DR13[b]	41	17	None
DR15	40	18	Urticaria upon contact with amidotrizoate; reacted to urography (1968) with discomfort, vomiting
DR16[c]	28	7	None
DR22	34	8	Itching after contact with amidotrizoate
DR23	54	30	None
DR26[d]	35	16	None
DR27	58	10	Urticaria upon contact with iodine-containing products
DR28	52	28	Rhinitis upon contact with amidotrizoate; no reactions to iohexol
DR30	47	25	None
DR31	40	7	None
DR32	38	15	Rhinitis, eczema upon contact with metrizoate and amidotrizoate; no reactions to iohexol
DR33	40	17	None
DR34[e]	36	15	None
DR35	42	21	Urticaria (face, neck) upon contact with amidotrizoate; no reactions to iohexol
DR37	46	3	None
DR39	35	0.5	None

[a]Reacts to wasp stings.
[b]Reacts to seafood (shrimps, crab meat), wasp stings; atopic predisposition.
[c]Reacts to birch and grass pollen, sulpha drugs; atopic disposition.
[d]Reacts to grass pollen, seafood, sulpha drugs.
[e]Reacted to penicillin with Quincke's edema.

scintillation counter. The ^{3}H-thymidine incorporation in antigen-treated cultures is expressed as mean △ counts per minute or stimulation index (SI):

SI = mean cpm in antigen-treated culture/cpm in control culture.

△ cpm = mean cpm in antigen-treated culture minus mean cpm in control culture.

Morphologic observations. The proliferative responses in 5-day cultures were evaluated by counting of lymphoblasts on cell smears as described previously (24). These evaluations were always performed by one and the same researcher and were always performed before the radioisotope analysis of lymphocyte proliferation.

Evaluation of LTT. The results of LTT were considered positive only if the mean cpm of antigen-treated cultures was more than twice the mean cpm of control cultures (SI > 2), and if the antigen-treated cultures contained twice as many lymphoblasts as the control cultures.

Preparation of iodinated serum proteins. An aqueous solu-

Table 3

Lymphocyte proliferation response to intravenously administered amidotrizoate, iohexol and potassium iodide in patients with hypersensitivity reactions

Code	Amidotrizoate[a]		Iohexol		KI		Tuberculin
	Δ-cpm	SI	Δ-cpm	SI	Δ-cpm	SI	SI
DR1	474	1.9	n.d.		918	6.4	30
DR3	1 175	2.2	105	1.0	n.d.	n.d.	6.5
DR14	365	2.1	134	1.4	400	2.2	288
DR17	15 210	2.7	461	1.1	2 643	1.3	6.9
DR19	−215	0.8	126	1.1	88	1.1	235
DR21	20	1.0	n.d.		2 523	4.7	301
DR24	1 898	1.3	n.d.		7 756	2.1	24
DR25	1 417	3.7	n.d.		2 958	4.8	291

[a]Maximum lymphocyte proliferation shown.
[b]Positive proliferative responses are underlined.
n.d. = not determined.

Table 4

In vitro proliferative lymphocyte responses to amidotrizoate, iohexol (DR12), and potassium iodide in patients without side effects associated with amidotrizoate urography

Code	No. of urographies	Amidotrizoate[a] Δ-cpm	SI	KI Δ-cpm	SI	Tuberculin SI
DR5	1	93	1.3	34	1.1	250
DR6	7	346	2.2	3 513	13	50
DR7	2	10 718	1.5	112 907	6.6	2.2
DR8	5	422	1.8	3 562	7.5	234
DR12[c]	6	1 129	2.0	2 469	3.1	9.8
DR18	3	−1 711	0.7	−1 195	0.8	n.d.

[a]Maximum lymphocyte proliferation shown.
[b]Positive proliferative responses underlined.
[c]Iohexol was used for urography and for LTT.
n.d. = not determined.

tion containing 14 mg I/ml as amidotrizoate or iohexol was irradiated in presence of 0.7 mg human gammaglobulin per ml with a dose of 1 or 0.2 Gy using a ^{60}Co Picker source, Hot-Pot-type, delivering about 1 Gy of gamma rays per second. A solution of human gamma globulin without radiographic contrast medium served as control. Dialysis of the irradiated solutions for 8 hours was repeated three times in a large volume of 0.9% of NaCl. Iodination of protein was also performed in a solution of the same composition (amidotrizoate) in the presence of 0.1 mM hydrogen peroxide and 0.2 mM ferrous sulfate.

Results

Previously exposed patients. The results of the lymphocyte proliferation assays in patients who had previously experienced various hypersensitivity reactions upon injection of amidotrizoate are presented in Table 3. Of 8 patients, only the lymphocytes from 2 patients, DR17 and DR25, exhibited a clear positive reaction to amidotrizoate in the LTT. Lymphocytes from patient DR3 showed only a weak proliferative response (SI = 2.2 with a 3-fold increase in the number of lymphoblasts). By contrast, a significant LTT reaction to iohexol did not occur in any of the patients tested. Patient DR25 underwent 10 consecutive urographies with amidotrizoate during 1976 to 1983, and on several of these occasions experienced Quincke's edema and urticaria. Also, patient DR17 experienced an anaphylactic reaction which required immediate cortisone treatment in association with urography in 1978. After injection of only 3 ml of amidotrizoate, there was a sharp fall in blood pressure accompanied by dyspnea; vomiting occurred and Quincke's edema of the throat and legs developed. Except for allergy to cats, this male patient had no previous history of hypersensitivity. As demonstrated for patient DR17, the response of sensitive lymphocytes to amidotrizoate was dose-dependent up to a maximum concentration (Fig. 1). However, the cytotoxic dose where inhibition of thymidine incorporation occurs was not attained in this experiment. For comparison, the lack of response exhibited by lymphocytes derived from patient DR18, who underwent uneventful urography, is shown in the same figure. The LTT responses from the other 6 patients without side effects associated with amidotrizoate urography were likewise negative (Table 4).

Table 5

In vitro lymphocyte proliferation responses to amidotrizoate, iohexol and potassium iodide in occupationally exposed nurses

Code	Subjective symptoms[a]	Years of exposure	Amidotrizoate[b] Δ-cpm	SI	Iohexol Δ-cpm	SI	KI Δ-cpm	SI
DR15	+	18	4 734[c]	3.2	n.d.	n.d.	1 299	4.9
DR22	+	8	−334	0.7	n.d.	n.d.	4 204	4.9
DR27	+	15	3 245	4.0	1 186	1.1	4 992	2.0
DR28	+	28	10 426	3.1	n.d.	n.d.	300	1.1
DR32	+	15	13 499	5.5	165	1.1	n.d.	n.d.
DR35	+	21	10 781	8.3	389	1.3	5 353	2.8
DR16	−	7	15 030	6.1	n.d.	n.d.	5 940	13
DR26	−	16	2 591	6.2	793	2.5	−72	0.8
DR30	−	25	2 565	8.2	177	1.5	266	1.2
DR31	−	7	4 012	4.0	n.d.	n.d.	1 070	1.7
DR33	−	17	5 293	4.5	341	1.2	n.d.	n.d.
DR37	−	3	4 756	3.0	68	1.0	2 299	6.1
DR9	−	>14	677	2.5	n.d.	n.d.	13 952	33
DR13	−	17	1 633	4.7	426	2.0	264	1.2
DR23	−	30	1 103	4.4	n.d.	n.d.	1 777	6.5
DR34	−	15	474	2.0	106	1.3	729	2.8
DR39	−	1	352	2.2	−12	1.0	n.d.	n.d.

[a]Presence (+) or absence (−) of subjective symptoms of hypersensitivity.
[b]Maximum lymphocyte proliferation is shown.
[c]Positive proliferative responses are underlined.
n.d. = not determined

Exposed nurses. Several nurses employed at the radiologic clinic at Danderyds Sjukhus had complained of hypersensitivity reactions associated with the handling of radiographic contrast media. In order to further explore the antigenic potential of these agents, we have studied the lymphocyte responses of 17 exposed nurses in this clinic. Table 5 summarizes the data obtained for amidotrizoate and iohexol. Eleven of the 17 nurses exhibited a positive LTT response to amidotrizoate, but none reacted to iohexol. Selected individual responses are shown in Fig. 2 as well as in Table 6. Lymphocytes from 2 amidotrizoate sensitive nurses (DR32 in Fig. 2; DR35 in Table 6) cross-reacted with the structurally closely related metrizoic acid and iothalamate. By contrast, no proliferation was obtained with the non-ionic agents iohexol, iopamidol or iopromide.

Unexposed controls. In Table 7 the LTT responses to amidotrizoate as well as to inorganic iodine are presented for healthy, unexposed individuals. The lymphocytes of 2 individuals (TL31, TL49), who had never been exposed to radiographic contrast media, responded to amidotrizoate. This raised the possibility of cross sensitization with respect to other iodine containing compounds. In addition, lymphocytes from several tested individuals gave a positive response to potassium iodide.

Antigenicity of iodo-proteins. The possibility that iodinated serum proteins formed by irradiation of iodinated contrast media was also investigated. However, a mixture of amidotrizoate and HSA irradiated with 1 Gy x-rays failed to elicit a proliferative response in lymphocytes from several patients whose lymphocytes responded to amidotrizoate (e.g. DR17, who had suffered from an anaphylactic reaction). However, in a few cases with a positive LTT reaction to amidotrizoate, proliferation occurred, but stimulation by the irradiated material was less than the response induced by amidotrizoate alone. Alternatively, iodinated protein mixtures were prepared by generating hydroxyl radicals with Fenton's reagent (hydrogen peroxide/ferrous iron) in an amidotrizoate/HSA solution. Again, the products formed were less active than the pure radiographic contrast medium (results not shown).

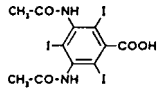
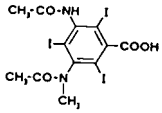
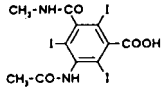
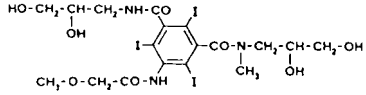
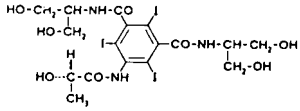
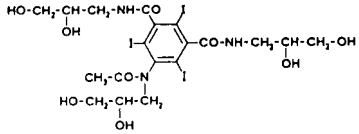
Lymphocyte proliferation induced by inorganic iodine. To investigate whether the proliferation induced in the lymphocytes from some patients by radiographic contrast media could be due to traces of inorganic iodine present in the commercial preparations, the LTT was also performed in the presence of potassium iodide. As shown in Tables 3, 4 and 5, there was no obvious correlation between the responses induced by contrast medium and those caused by iodide.

Discussion

In the present investigation, we have shown that ionic radiographic contrast media, such as amidotrizoate and metrizoate, possess antigenic properties. For instance, lymphocytes from 2 out of 8 sensitive patients, as well as lymphocytes from all but one nurse with subjective hypersensitivity reactions, were positive to amidotrizoate in the LTT. In contrast to this finding, similar reactions did not occur with iohexol or with the other non-ionic contrast media tested. These results obtained in vitro are in agreement with general clinical experience (8). The fact that the

Table 6

Lymphocyte proliferation in response to 6 different radiologic contrast media in an occupationally exposed nurse (DR35) experiencing urticaria while working with amidotrizoate

Antigen in culture	Structure	Lymphocyte proliferation Δ-cpm	SI	Lymphoblasts
Amidotrizoate		10 781	8.3	+
Metrizoate		3 396	3.6	+
Iothalamate		4 787	4.7	+
Iopromide		1 854	2.4	0
Iopamidol		168	1.1	0
Iohexol		389	1.3	0

majority of the amidotrizoate sensitive patients lacked specific memory cells supports a multifactorial etiology for these side effects (1). The occurrence of pseudoallergic reactions is a common finding; in our previous investigations on penicillin allergy, a considerable percentage of the patients with a history of hypersensitivity lacked penicillin-specific memory cells as revealed by LTT. Only those patients with specific memory cells reacted upon subsequent provocation (21, 23). GIRARD & GAMBA (11) obtained a positive LTT response in 40 out of 54 patients who had experienced side reactions from radiographic contrast media. The reason for the much higher incidence of LTT positive reactions in the material of GIRARD & GAMBA can probably be ascribed to the fact that monitoring of lymphocyte proliferation solely by incorporation of labeled thymidine, as performed by those investigators, gives false positive readings. In our methodology this is avoided by scoring lymphocyte morphology in parallel (21, 24).

The present study further demonstrates, that occupational exposure to ionic contrast media may result in sensitization, and that cross reactions between the structur-

Table 7

In vitro lymphocyte proliferation responses to amidotrizoate, potassium iodide, and to tuberculin in control subjects not previously exposed to amidotrizoate

Code	Amidotrizoate[a] Δ-cpm	SI	KI Δ-cpm	SI	Tuberculin SI
DR10	161	1.0	4 909[b]	3.0	19
DR11[c]	78	1.1	1 317	2.9	339
RB	285	2.6	127	1.0	91
HP10	67	1.5	397	3.5	795
HP19	209	1.3	n.d.		514
UH	5	1.0	11	1.0	326
TL31	3 232	4.8	4 644	6.6	40
TL45	507	2.7	680	3.3	11
TL49	3 625	7.3	n.d.		5.3

[a]Maximum lymphocyte proliferation is shown.
[b]Positive proliferative responses are underlined.
[c]Hypersensitivity to nickel, shrimps.
n.d. = not determined.

ally closely related amidotrizoate and metrizoate may occur. The fact that 5 nurses experienced subjective symptoms of hypersensitivity while handling radiographic contrast media, as well as the high incidence of positive LTT responses to amidotrizoate in this occupationally exposed group, gives further evidence of the antigenic potential of this type of iodinated compounds. The positive LTT response obtained in 2 individuals who had not been previously exposed to these agents, may be interpreted as cross sensitization involving previous contact with structurally similar antigens in the patient's environment. Similar findings as to the occurrence of positive LTT reactions to radiographic contrast media in previously unexposed individuals have been reported by GIRARD & GAMBA (11). Since hypersensitivity towards inorganic iodine was not present in any of the patients studied, the significance of the positive LTT reactions to inorganic iodide found in individuals sensitive to amidotrizoate as well as in controls remains obscure. There was no obvious correlation between the responses induced by contrast medium and those caused by iodide.

It has been shown, that many adverse reactions to radiographic contrast media resemble IgE-mediated allergic manifestations (6, 11). Further, patients who have previously experienced side effects from such agents are at greater risk for severe reaction upon repeated exposure than individuals who have undergone uneventful radiography (2, 19). In some published clinical reports it appears that an allergic etiology has been involved (16). Positive (4, 6, 13, 16, 25, 26) as well as negative (7, 15, 27) results from attempts to identify antibodies specific for iodinated contrast media have been published. In a short communication SWEENEY & KLOTZ (25) identified IgE antibodies in 11 out of 26 patients with a positive clinical history of adverse reactions to radiographic contrast media and in 5 out of 5 patients with an immediate positive clinical reaction. SIEGLE et al. (20) have recently been able to induce the formation of polyclonal and monoclonal antibodies with diatrizoate coupled to albumin in rabbits and mice. The direct release of histamine has been advanced as an alternative hypothesis (17).

We have previously proposed, that antigenic iodo-proteins formed during radiography may play a role in eliciting hypersensitivity reactions in previously sensitized individuals (18). Although such a mechanism cannot be excluded in the case of certain rare and unpredictable reactions, this hypothesis is not supported by the present data.

Conclusion. The LTT technique may under certain conditions be used as a diagnostic tool for the screening of immune reactions to radiographic contrast media. The significance of amidotrizoate specific lymphocytes seen in some exposed but symptom-free persons remains to be elucidated.

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