

Original article

Cross-reactivity patterns of T cells specific for iodinated contrast media

Marianne Lerch, MD,^{a,*} Monika Keller, PhD,^{a,*} Markus Britschgi, PhD,^a Gisele Kanny, MD, PhD,^b Valerie Tache,^a Daphne A. Schmid, PhD,^a Andreas Beeler, MSc,^a Basil O. Gerber, PhD,^a Michael Luethi,^a Andreas J. Bircher, MD,^c Cathrine Christiansen, MSc,^d and Werner J. Pichler, MD^a Bern and Basel, Switzerland, Nancy, France, and Oslo, Norway

Background: Approximately 3% of patients exposed to iodinated contrast media develop delayed hypersensitivity reactions.

Objective: We wanted to better understand the molecular basis of contrast media cross-reactivity.

Methods: Cross-reactivity was assessed by skin testing and measurement of T-cell activation (CD69 upregulation) and proliferation (³H-thymidine uptake, 5,6-carboxyfluorescein diacetate succinimidyl ester staining) of PBMCs, T-cell lines, and T-cell clones of 2 patients with delayed hypersensitivity reactions to iohexol and iomeprol, respectively. Thirteen different contrast media and potassium iodide were compared. **Results:** Skin testing and analyses of PBMCs, T-cell lines, and clones showed broad cross-reactivity in both patients. Broad as well as more restricted cross-reactivity patterns were found in iohexol-specific and iomeprol-specific CD4⁺ T-cell clones, whereas 1 iomeprol-specific CD8⁺ T-cell clone showed no cross-reactivity at all. The reactivity to equimolar concentrations of iohexol and its dimer iodixanol was very similar, suggesting that the dimer was not more stimulatory than its monomer. Consistently low reactivity to iobitridol was found in both patients, but never to iodide. A frequency analysis of contrast medium-specific peripheral T cells gave values between 0.6 % (iomeprol) and 0.05 % (iobitridol).

Conclusion: Clinically observed cross-reactivity between different contrast media is a result of the presence of contrast media-specific T cells, some of which show a broad cross-reactivity pattern. Iodide ions, known to be present at low concentration in contrast media solutions, do not seem to be the causative moiety.

Clinical implications: Detailed *in vitro* analysis might help identify noncross-reactive contrast media. (J Allergy Clin Immunol 2007;119:1016-1022.)

Key words: Contrast media, hypersensitivity, T-cell line, T-cell clone, cross-reactivity, precursor frequency

Iodinated contrast media (CMs) have been indispensable diagnostic tools since the 1930s. They are used with a frequency of more than 75 million applications per year. Normally, they are well tolerated, but adverse reactions may occur. These can be divided according to time of appearance: immediate-type reactions that occur within 1 hour and delayed-type reactions that occur from 1 hour to 1 week later. Immediate hypersensitivity reactions to CMs seem to involve mast cell degranulation and resemble anaphylactic reactions. They have a frequency of approximately 0.7% to 3.1% in patients receiving non-ionic CMs.^{1,2} Delayed hypersensitivity reactions occur with a frequency of 1% to 3%, and the incidence is higher after previous adverse reactions to CMs.^{3,4} Delayed-type reactions usually manifest themselves as mild maculopapular exanthems. Rarely, more severe skin eruptions, such as bullous rashes, occur. Positive skin patch tests and lymphocyte transformation tests (LTTs) suggest a T-cell-mediated process.^{3,5,6}

All CMs on the market have a very similar chemical structure consisting of monomers or dimers of heavily substituted, tri-iodinated phenyl rings with different side chains (see this article's Fig E1 in the Online Repository at www.jacionline.org). This similarity of the chemical structure poses a clinical problem to further diagnostic procedures if an immune-mediated reaction has occurred after a previous CM administration¹: for example, will a delayed hypersensitivity reaction to the CM "A" be followed by a similar reaction to an alternative CM "B"? To understand better the molecular basis of cross-reactivity, we performed a detailed study of the cross-reactivity patterns of 2 well defined patients with previous delayed reaction to iohexol and iomeprol, respectively. First, we investigated the cross-reactivity to various CMs by patch and intradermal test. Second, we investigated the ability of different CMs to stimulate the PBMCs and T-cell lines (TCLs) from one of these patients and determined the frequencies of peripheral T cells with specificity for different CMs

From ^athe Clinic for Rheumatology and Clinical Immunology/Allergology, Inselspital, University of Bern; ^bthe Department of Internal Medicine, Clinical Immunology and Allergology, University Hospital, Nancy; ^cthe Allergy Unit, Department of Dermatology, University Hospital, Basel; and ^dResearch and Development, GE Healthcare, Oslo.

*These authors equally contributed to this work.

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Reprint requests: Werner J. Pichler, MD, Clinic for Rheumatology and Clinical Immunology/Allergology, PKT2 D572, Inselspital, CH-3010 Bern, Switzerland. E-mail: werner.pichler@insel.ch.

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Abbreviations used

CFSE: 5,6-Carboxyfluorescein diacetate succinimidyl ester
 CM: Contrast medium
 LTT: Lymphocyte transformation test
 NMFI: Normalized mean fluorescent intensity
 TCC: T-cell clone
 TCL: T-cell line
 TCR: T-cell receptor
 TT: Tetanus toxoid

(precursor cell frequency). Third, we generated T-cell clones (TCCs) from the PBMCs of the 2 patients and studied the cross-reactivity pattern of these clones in detail. The data generated show that cross-reactivity can be detected by different methods, that cross-reactivity can be quantified by determining precursor cell frequency to various CMs, and that some TCCs show broad cross-reactivity, whereas others are not cross-reactive at all.

METHODS

Patients

Patient 1, woman, 71 years. A generalized rash developed 18 hours after an angiography with iohexol.

Patient 2, man, 60 years. Seventy-two hours after iomeprol injection for coronary angiography, the patient, who had previously been exposed to an unknown CM, developed a generalized maculopapular exanthem.

See this article's [Online Repository](#) for additional methods information concerning this and the following sections.

Skin tests

Skin prick tests, intradermal tests, and patch tests with CMs were performed according to a previously published protocol.^{5,7,8}

LTT and generation of CM-specific TCLs and TCCs

Lymphocyte transformation tests were performed by stimulating PBMCs with different concentrations of CM as indicated. After 6 days, 0.5 μ Ci ³H-thymidine was added to the cell culture, cells were harvested after 10 to 14 hours, and ³H-thymidine uptake was determined on a scintillation liquid-free β -counter.⁹ TCLs were generated by stimulating PBMCs with the indicated CM and a restimulation after 10 days with irradiated autologous PBMC and the respective CM.⁹⁻¹² TCCs were generated from TCLs by limiting dilution technique as described elsewhere.^{10,11}

T-cell proliferation assay

Contrast media specificity of T cells was tested by incubating 5×10^4 cells together with irradiated autologous antigen-presenting cells in the presence or absence of CM. After 48 hours, 0.5 μ Ci ³H-thymidine was added and incorporated radioactivity was measured.

Characterization of TCCs

Fluorochrome-labeled antihuman CD3, CD4, and CD8 (BD, Franklin Lakes, NJ) and T-cell receptor (TCR)–V β chain—specific (Immunotech, Marseille, France, and Serotec, Oxford, United Kingdom)^{9,11,12} mAbs were used to phenotype the TCCs. Flow cytometry was performed on a BD FACSCanto Flow Cytometer (BD Biosciences, San Jose, Calif). Monoclonality of selected TCCs

(AD Om27, BS Iom4*1/76, BS Iom3*0.3/28) was determined by sequencing of the V α and V β regions.¹³

CD69 upregulation assay

PBMCs were incubated with the indicated CM, tetanus toxoid (TT), or potassium iodide. CD69 expression on T cells was monitored by flow cytometry after 18, 36, and 72 hours of incubation by using antihuman CD69, CD4, CD8, and CD3 mAbs (BD Pharmingen, Franklin Lakes, NJ). Results are expressed as normalized mean fluorescent intensity (NMFI), which is the percentage of positive lymphocytes multiplied by the corresponding mean fluorescent intensity values.¹⁴

5,6-Carboxyfluorescein diacetate succinimidyl ester–based proliferation assay

To evaluate T-cell proliferative responses to different CMs, 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE) staining was used as previously described.¹⁵ CM-stimulated, CFSE-labeled PBMCs were analyzed with a BD FACSCanto Flow Cytometer by using antihuman CD3, CD4, and CD8 mAbs. Data were analyzed by using FlowJo 7.1.1 (Tree Star Inc, Ashland, Ore), and precursor frequencies were calculated by using the Proliferation Wizard module in ModFit LT Macintosh software (Verity Software House, Topsham, Me).

RESULTS

Skin tests

Table 1 shows that the investigated patients were skin test–positive to a panel of different CMs. Because patient 2 was patch test–negative and only weakly intradermal test–positive to ioxaglate, this CM was chosen for provocation. However, injection of 31 mL of this CM resulted in the reappearance of a rash, illustrating that even a CM causing only a weakly positive skin test may elicit a reaction on provocation.

Proliferation assays and CD69 upregulation

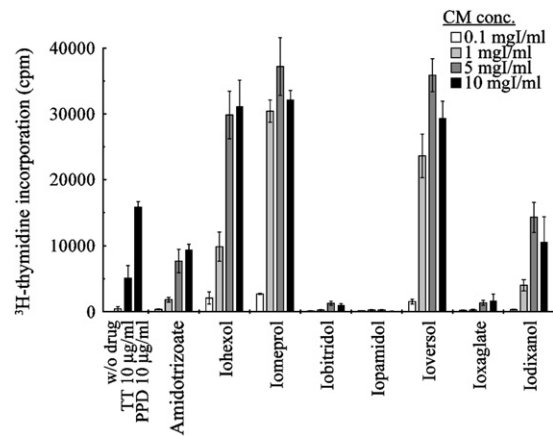
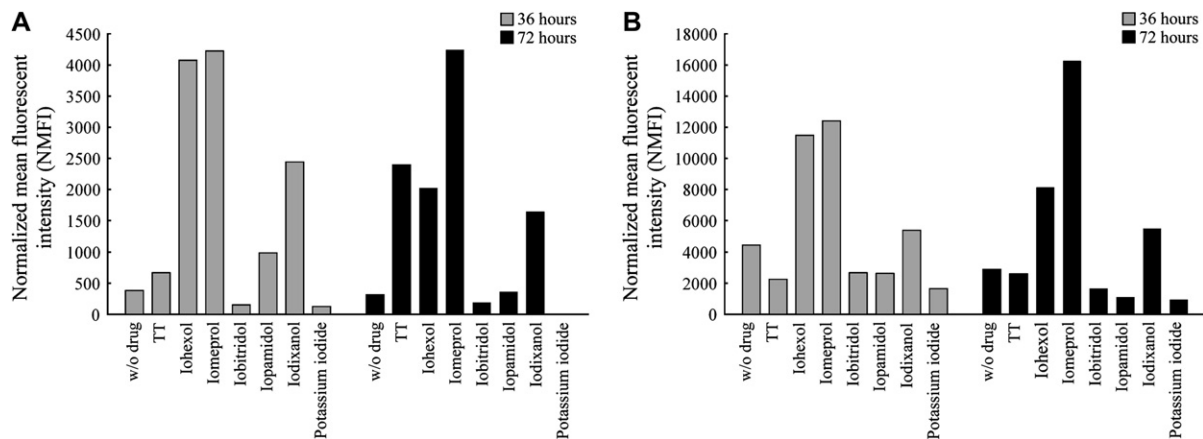
The 2 patients selected had strongly positive LTTs to the culprit CM. The proliferation of PBMC from patient 1 and 2 was 20-fold and 70-fold higher, respectively, in the presence of CM (1 mg/mL) compared with the control culture. PBMCs from patient 2 were tested for cross-reactivity using 8 different CMs (**Fig 1**). As expected from the results of the skin testing, the PBMCs of patient 2 responded to several different CMs to which he had never been exposed. However, no or only a marginal proliferation was seen to iobitridol, iopamidol, and ioxaglate despite positive skin tests (**Table I**).

Alternatively, activation of PBMC after stimulation with drugs can be determined by measuring upregulation of the early activation marker CD69 (Beeler et al, unpublished data, January 2006). In **Fig 2**, CD69 upregulation on PBMCs of patient 2 after stimulation with CM is shown as NMFI, which accounts not only for the number of positive cells but also for the density of CD69 molecules expressed on the cell surface. A significant upregulation of CD69 on CD4⁺ T cells (**Fig 2, A**) was observed when PBMCs of patient 2 were stimulated with different CMs. The data shown as NMFI in **Fig 2** corresponded to 1.8% of CD4⁺ T cells for iohexol, 1.9% for iomeprol, and 1.3%

TABLE I. Skin test results for patients 1 and 2

Skin test substances	Patient 1 (iohexol reactor)		Patient 2 (iomeprol reactor)		IDT
	Prick	Patch	Prick	Patch	
Amidotrizoate	–	–	–	–	–
Ioxithalamate	–	+	–	–	+
Iohexol	–	+	–	+	+
Iopentol	–	+	–	+	+
Iomeprol	ND	ND	–	+	+
Iopromide	–	+	–	+	+
Iobitridol	–	+	–	+	–
Iopamidol	–	+	–	+	+
Ioversol	–	+	–	+	+
Ioxaglate	–	+	–	–	+
Iotrolan	ND	ND	–	–	–
Iodixanol	–	+	–	+	+

IDT, Intradermal test; ND, not done.

**FIG 1.** Lymphocyte transformation test of patient 2. Proliferation of PBMCs from patient 2 measured by ^3H -thymidine incorporation (counts per minute) after stimulation with different concentrations of CMs (0.1, 1, 5, 10 mg/mL, respectively) is shown. The results are representative of 2 independent experiments. The error bars represent 1 SEM. *conc.*, Concentration; w/o, without; PPD, tuberculin purified protein derivative.**FIG 2.** Upregulation of the early activation marker CD69 on PBMCs of patient 2 after stimulation with different skin test-positive CMs. **A**, The percentage of CD4^+ cells showing CD69 upregulation is depicted as NMFI. **B**, CD69 upregulation on CD8^+ cells. Shown is 1 representative experiment of 3 performed. w/o, Without.

for iodixanol, respectively. However, no upregulation was found in the presence of iobitridol, iopamidol, or potassium iodide. Similar results were found for CD8^+ T cells (Fig 2, B): iohexol, iomeprol, and iodixanol gave a CD69 upregulation of 3.2%, 3.3%, and 0.6%, respectively, whereas iobitridol, iopamidol, and potassium iodide gave no CD69 upregulation above background. PBMCs of 2 TT reactive controls never exposed to CM did not show any upregulation of CD69 in the presence of the tested CM at 6 different time points (data not shown).

Precursor frequency

To determine the number of peripheral T cells with specificity for different CMs (precursor frequency), we used CFSE staining assays.¹⁶ This method relies on the color intensity of the CFSE-labeled PBMCs, which is reduced by 50% with each cell division. As shown in

Fig 3, the CFSE-fluorescence intensity of the CD4^+ and CD8^+ T cells decreased significantly in cultures stimulated with several of the investigated CMs compared with the unstimulated control. From these flow-cytometric data, the precursor frequencies of CM-specific CD4^+ and CD8^+ T cells were calculated. As shown in Table II, the frequency of peripheral CD4^+ and CD8^+ T cells with specificity for iomeprol, iohexol, and iodixanol was in the range of 0.13% to 0.6% and 0.07% to 0.4%, respectively. The very low frequency of T cells with specificity for iobitridol and iopamidol was again confirmed (precursor frequencies 0.05% and 0.02%, respectively).

CM-specific TCLs

Cross-reactivity analyses were also conducted using 2 TCLs generated from PBMCs of patient 2 through stimulation with iomeprol and iodixanol, respectively. As

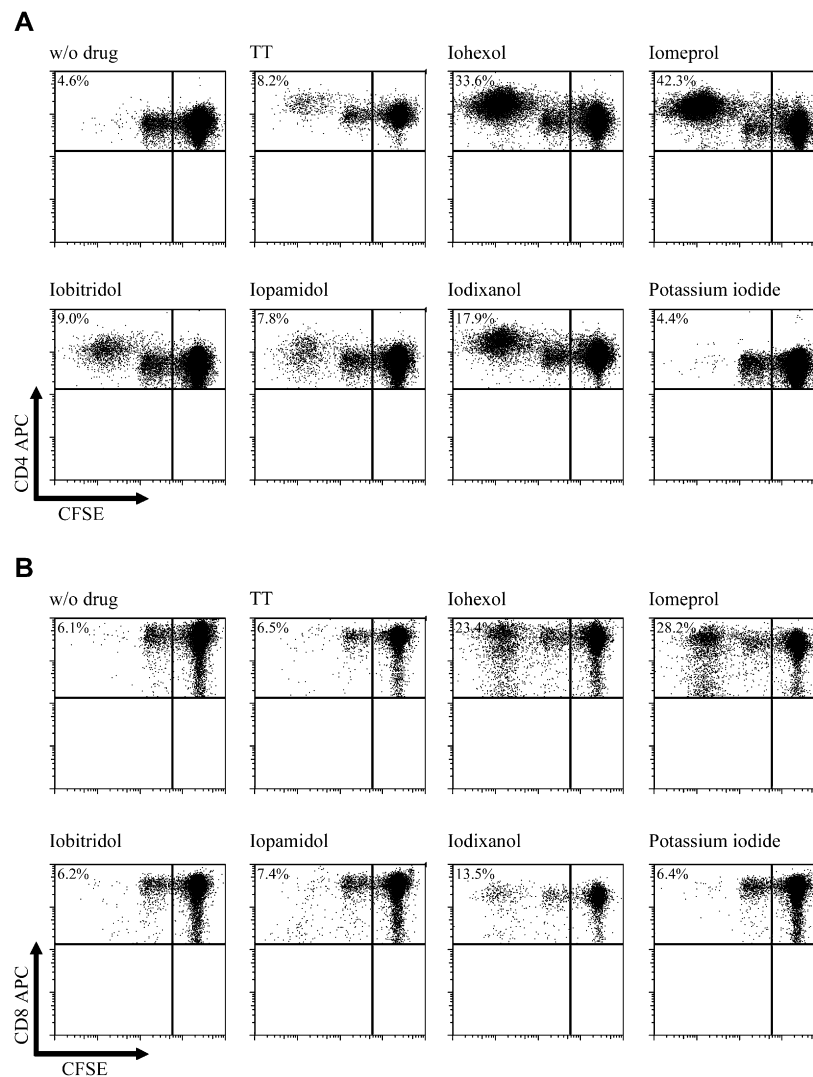


FIG 3. CFSE intensity distribution analysis of PBMCs of patient 2 after stimulation with different CMs for 7 days. Similar results were obtained for CD4⁺ (A) as well as for CD8⁺ T cells (B). Shown is 1 representative experiment of 2 performed. *w/o*, Without.

shown in Fig 4, high stimulation indices were found for a large number of the CMs tested. Cross-reactivity patterns were very similar for the 2 TCLs, in spite of the fact that they were generated through stimulation with 2 different CMs. Differences were seen for iobitridol and iotrolan that were weakly stimulatory for the iomeprol-generated TCL but failed to stimulate the iodixanol-generated TCL.

Cross-reactivity of TCCs

The data shown demonstrate that the polyclonal T-cell populations found in the PBMCs or TCLs of patient 2 showed a high degree of cross-reactivity. To perform an extensive analysis of cross-reactivity patterns of single CM-specific T cells, we generated TCCs from an iohexol-stimulated TCL of patient 1 and from iomeprol-stimulated TCLs of patient 2.

From the TCL of patient 1, 10 iohexol-specific CD4⁺ TCCs were obtained. Seven of these were analyzed in

detail. From TCLs of patient 2, we generated 87 iomeprol-specific TCCs. Of these, 7 CD4⁺ TCCs as well as 3 CD8⁺ TCCs were further analyzed.

Sequencing showed that the 3 CD8⁺ TCCs expressed the same TCR-V α and V β and hence were probably derived from the same T cell. The phenotypic characterization of the CD4⁺ TCCs showed distinct TCR-V β chains, confirming their different clonal origin. Thus, in patient 2, the CD4 response seems to be more polyclonal, because T cells with different TCR-V β reacted, whereas the CD8 response was more restricted.

When performing cross-reactivity assays with the iohexol-specific TCCs of patient 1 with 11 different compounds, 2 patterns of cross-reactivity were found (Fig 5, A). Two of the tested iohexol-specific TCCs reacted with iohexol, iopentol, iomeprol, and amidotrizoate (TCC Om3 and Om7), whereas 4 clones reacted with iohexol, iopentol, and iomeprol and in addition with

TABLE II. CFSE intensity distribution analysis of PBMCs from patient 2

	Precursor frequency (Δ %) [‡]
CD4 ⁺ *	
Iohexol 5 mgI/mL	0.343
Iomeprol 5 mgI/mL	0.600
Iobitridol 5 mgI/mL	0.047
Iopamidol 5 mgI/mL	0.019
Iodixanol 5 mgI/mL	0.131
TT 10 μg/mL	0.042
KI 100 ng/mL	0.000
CD8 ⁺ †	
Iohexol 5 mgI/mL	0.192
Iomeprol 5 mgI/mL	0.405
Iobitridol 5 mgI/mL	0.000
Iopamidol 5 mgI/mL	0.008
Iodixanol 5 mgI/mL	0.074
TT 10 μg/mL	0.006
KI 100 ng/mL	0.007

KI, Potassium iodide.

*Without drug: 0.0019%.

†Without drug: 0.0045%.

[‡]Δ: Given are values after subtraction of background.

ioxaglate, iotrolan, and iodixanol (TCCs Om4, 27, 30, and 40). Iopromide, iobitridol, and iopamidol did not stimulate any of the TCCs raised against iohexol. The CD4⁺ TCCs of patient 2 generated with iomeprol showed a broad cross-reactivity with different CMs including both ionic CMs, like amidotrizoate, as well as nonionic monomers, like ioversol (shown for 1 TCC in Fig 5, B). In contrast, the CD8⁺ TCC Iom3*0.3/28 reacted only with iomeprol (Fig 5, B).

To understand better the molecular basis of cross-reactivity, we generated dose-response curves with iohexol, iodixanol, and iomeprol by using 2 iodixanol-specific TCLs from patient 2. As shown in Fig 6, both iohexol and iodixanol produced an equal proliferation if the same molar concentrations were used.

DISCUSSION

Cross-reactivity is a clinically important problem,¹⁷ in particular for iodinated CMs, which are all based on the same core structure, a tri-iodinated phenyl ring and similar hydroxylated side chains. It is impossible at present to predict which alternative CM can be safely used in a patient with previous late hypersensitivity reaction after CM exposure. Skin tests seem to have a rather low sensitivity for evaluation of alternative CM, because it has been reported that close to 50% of patients undergoing re-exposure with a skin test-negative CM had a relapse of the skin eruption.¹⁸ Furthermore, prophylactic treatment regimens¹⁹⁻²¹ might be of limited value. This makes it difficult to choose an alternative CM in a patient with CM allergy.

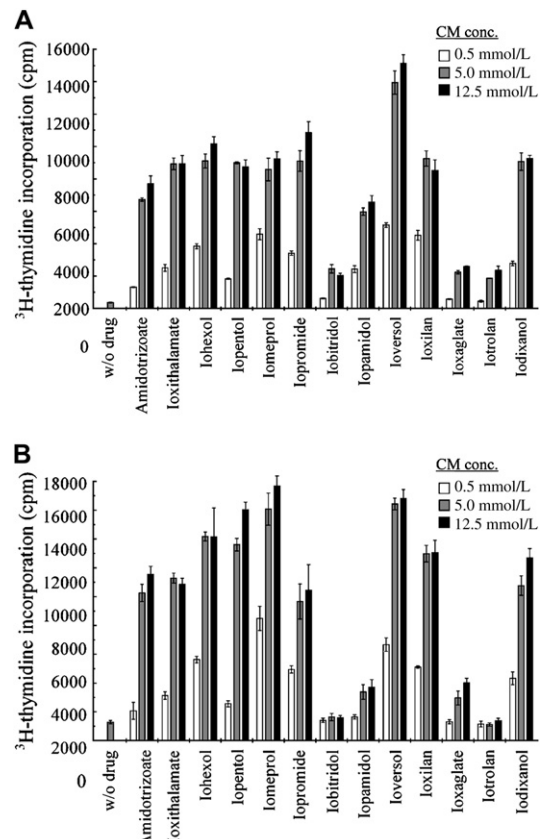


FIG 4. Cross-reactivity pattern of 1 iomeprol-specific (A) and 1 iodixanol-specific (B) TCL of patient 2 after 14 days and restimulation with phytohemagglutinin. The results are representative of 2 independent experiments. Note that some weakly stimulatory CMs (iobitridol, iotrolan) were negative in the iodixanol-stimulated TCL, but not in the iomeprol-stimulated TCL. The error bars represent 1 SEM. conc., Concentration; w/o, without.

In this study, we used 2 clinically well characterized patients to investigate in detail the cross-reactivity of various CMs. Intradermal and patch tests as well as proliferation assays with PBMC, TCLs, and TCCs were used. We investigated whether CD4⁺ and CD8⁺ T cells were reacting, and quantitated the reactivity by different means. Our results indicate that cross-reactivity is based on an extensive but variable cross-reactivity of single T cells, but even when extensive cross-reactivity patterns are observed, some CMs give low or undetectable responses.

The *in vivo* investigation already demonstrated a broad cross-reactivity pattern. In patient 2, both patch and intradermal tests were used with good correlation between the 2 tests. However, 3 compounds differed, being either positive in patch or intradermal tests. Possibly, even 1 positive result in these *in vivo* tests may already be an indication of cross-reactivity, because intravenous provocation with the patch test-negative but intradermal test-positive CM ioxaglate resulted in reappearance of the exanthem.

This *in vivo* cross-reactivity could be reproduced *in vitro*. The proliferation assays with PBMCs from patient 2 revealed a broad cross-reactivity to various CMs, but no

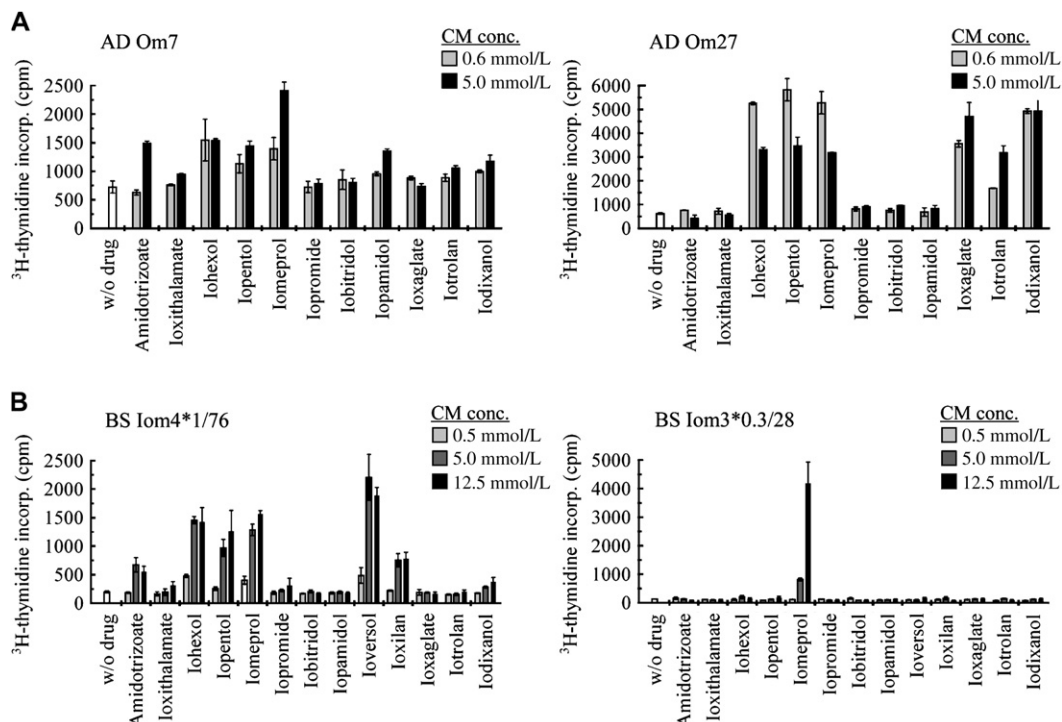


FIG 5. Cross-reactivity patterns of selected iohexol-specific and iomeprol-specific TCCs. In patient 1, 2 different patterns were found. TCC Om7 represents 1 of 2 iohexol-specific TCCs with a narrow cross-reactivity pattern, whereas TCC Om27 represents a specific TCC with a broad cross-reactivity pattern (A). In patient 2, the CD4⁺ TCC 4*1/76 showed a broad cross-reactivity pattern. In contrast with this, the CD8⁺ TCC 3*0.3/28 reacted only with iomeprol (B). *conc.*, Concentration; *incorp.*, incorporation; *w/o*, without.

or very moderate reactivity to iobitridol, iopamidol, and ioxaglate. TCLs raised against both the culprit CM iomeprol and the skin test–positive iodixanol confirmed this broad cross-reactivity at least for the strongly stimulatory CMs, because strong responses were detected to 10 of 13 and 9 of 13 CMs, respectively. However, the weakly stimulating CM differed regarding the 2 TCLs, because the iomeprol-stimulated TCLs could be stimulated with all 3 less stimulatory CMs as well, albeit only moderately, whereas the iodixanol-stimulated TCLs were still not reacting to iobitridol and iotrolan, and showed only a moderate response to iopamidol. This suggests that both TCLs are able to be stimulated by the majority of CMs, but that the weakly stimulatory CM can be detected only if the TCL is generated through stimulation of PBMCs with the agent that caused the clinical reaction. Using CD69 upregulation as an alternative method, we found similar results, with CD4⁺ as well as CD8⁺ T cells reacting to some but not all CMs.

It has been hypothesized that the probability of cross-reactivity is related to the precursor frequency of T cells able to react with different compounds.²² To investigate this in more detail, we attempted to define the precursor frequency by using CFSE staining. CFSE staining, which—in contrast with CD69 upregulation assays—does not detect activated cells in general, but exclusively proliferating T cells, revealed overall a frequency of as much as 0.5% CM-specific CD4⁺ and CD8⁺ T cells, respectively.

Again, a clear distinction between the different CM was observed, with no reactivity to iobitridol. Indeed, iobitridol was consistently negative in all the different assays using PBMCs, TCLs, and even TCCs, and seems therefore to be the best candidate CM if patient 2 needs a new CM exposure. This observation would also correlate with the observation by Vernassiere et al¹⁸ that iobitridol was negative in a provocation test in a patient with iodixanol allergy.

One commonly raised question is to what extent iodine itself is an allergen. Potassium iodide that is known to be a contaminant of commercial CM solutions was consistently negative in patch tests and also in precursor frequency analyses. Thus, our data support the concept that iodide by itself is not an immunogenic compound recognized by T cells.²³ Also, the positive skin test reactions to the disinfectant povidone iodine, reported in some patients with CM allergy, are probably mostly irritative in nature,^{24–26} and the same may apply for the positive skin test with the iodide/iodine (Lugol's) solution in patient AD.

From both patients 1 and 2, CD4⁺ TCCs could be generated that reacted with the eliciting drug and to a variable degree with other CMs. The TCCs of patient 1 revealed a broad cross-reactivity pattern as well as a more limited cross-reactivity. The analysis of CD4⁺ TCCs of patient 2 showed reactivity to a quite large spectrum of CMs. In contrast with this, a CD8⁺ TCC of patient 2 was found to react only with the eliciting CM iomeprol, but not with any other of the 12 CMs tested.

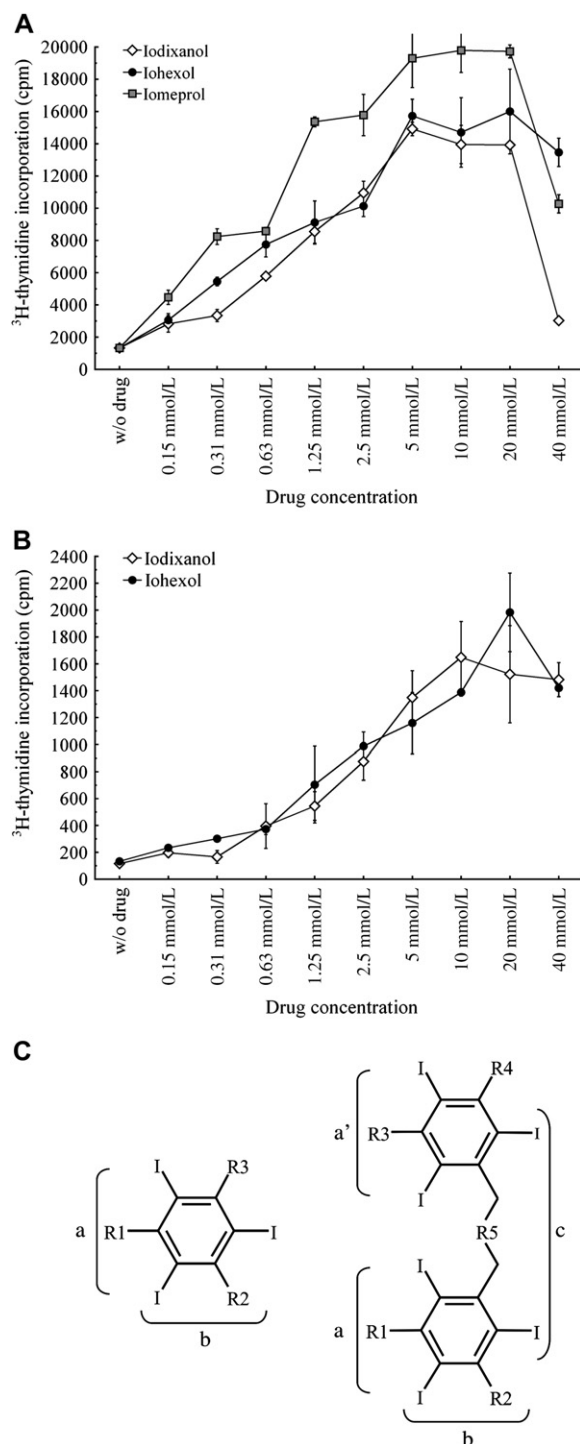


FIG 6. Dose-response of iodixanol-specific TCLs (**A** and **B**). In **C**, iohexol and its dimer iodixanol are schematically represented: the TCR might recognize the iodixanol as shown in *a*, *b*, or *c*. Because equimolar concentrations stimulated the TCLs very similarly, the dimeric structure does not seem to be important for TCR recognition (no recognition as shown in *c*). w/o, Without.

Our data do not definitively prove causality between T-cell reactivity and the clinical picture elicited. However, the congruency of skin tests and clonal results presented supports the concept that *in vivo* cross-reactivity is a result of a more or less extensive cross-reactivity of some CD4⁺ T cells, reacting with the eliciting drug as well as with other CMs. On the other hand, the reactivity of some CD8⁺ TCCs may be very restricted. This difference in cross-reactivity pattern of CD4⁺ and CD8⁺ cells needs to be substantiated in further studies.

Some information about how CMs are recognized by T cells on a molecular level was revealed by the comparison of the structurally related iohexol (monomer) and iodixanol (dimer): as equimolar concentrations of these CMs showed the same stimulatory potential, the TCR seems to recognize the iohexol structure in both compounds (viewpoint *a* or *b* in Fig 6, C), but not the dimeric structure or its bridging moiety (viewpoint *c* in Fig 6, C). These data show that a dimer is not more stimulatory than a monomer in an already sensitized individual.

In conclusion, the clinical consequences of our results might be the following:

- Cross-reactivity is common in CM-sensitized individuals, as has previously been pointed out.
- Performing detailed *in vivo* and *in vitro* analysis, one can identify noncross-reactive CMs. In this context, TCLs generated from the PBMCs of the previous reactor might be helpful screening tools.
- Contrast media reactivity is most likely not related to iodide/iodine hypersensitivity.
- The CM that caused the clinical symptoms should be identified and must be avoided. This CM is stimulating the complete T-cell response (CD4⁺ and CD8⁺) most vigorously, as in patient BS the drug-specific CD8⁺ T cells were strongly reacting with the eliciting CM, but noncross-reactive with other CMs. Such strong reactions of CD8⁺ T cells are of potential interest, because CD8⁺ T cells are known to be responsible for more severe allergic drug reactions.

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