Characterization of the T-Cell Response in a Patient with Phenindione Hypersensitivity

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ABSTRACT

The oral anticoagulant phenindione [2-phenyl-1H-indene-1,3(2H)-dione] is associated with hypersensitivity reactions in 1.5 to 3% of patients, the pathogenesis of which is unclear. We describe a patient who developed a severe hypersensitivity reaction that involved both the skin and lungs. A lymphocyte transformation test showed proliferation of T-cells from the hypersensitive patient, but not from four controls on exposure to phenindione in vitro. Drug-specific T-cell clones were generated and characterized in terms of their phenotype, functionality, and mechanism of antigen presentation. Forty-three human leukocyte antigen class II restricted CD4+ T-cell clones were identified. T-cell activation resulted in the secretion of interferon-γ and interleukin-5. Five of seven clones proliferated with phenindione alone, whereas two clones also proliferated with 2-phenylindene. Certain T-cell clones were also stimulated by R- and S-warfarin; computer modeling revealed that warfarin can adopt a phenindione-like structure. Phenindione was presented to T-cells via two pathways: first, bound directly to major histocompatibility complex and second, bound to a processed peptide. Our data show that CD4+ T-cells are involved in the pathophysiology of phenindione hypersensitivity. There may be cross-sensitivity with warfarin in some phenindione hypersensitive patients.

Phenindione [2-phenyl-1H-indene-1,3(2H)-dione], an indanedione derivative, is an oral anticoagulant with similar clinical indications as warfarin. Phenindione was introduced in the early 1950s, but its usage has remained low because of its potential to cause hypersensitivity reactions in 1.5 to 3% of individuals. The skin is the most common organ to be affected, although occasionally extracutaneous manifestations may be predominant. The reactions vary in severity and occasionally can be fatal (Mohamed, 1965; McMenamin et al., 1976). Clinical symptomatology, the delayed onset of the reaction and the demonstration of phenindione-specific lymphocyte proliferation (Potier et al., 1975), are all suggestive of an immune pathogenesis; however, the nature of the immune response and how phenindione is presented in an antigenic form has not been delineated.


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ABBREVIATIONS: MHC, major histocompatibility complex; HLA, human leukocyte antigen; IL, interleukin; SI, stimulation index; ELISA, enzyme-linked immunosorbent assay; IFN-γ, interferon-γ.
and the T-cell receptor (Schnyder et al., 1997, 2000). Detailed cross-reactivity studies have shown that antigen specificity is determined by the avidity of the drug for the MHC T-cell receptor complex and the T-cell receptor density (von Greyerz et al., 2001; Depta et al., 2004). Further studies using structurally unrelated compounds such as lidocaine, mepivacaine, lamotrigine, carbamazepine, phenobarbital, and p-phenylenediamine have also shown that specific T-cells can be stimulated through a noncovalent interaction of a drug with the MHC and the T-cell receptor (Zanni et al., 1998, 1999; Hashizume et al., 2002; Naisbitt et al., 2003a,b).

In this study, we describe a patient who developed a severe hypersensitivity reaction to phenindione. We have investigated the pathophysiology of the hypersensitivity reaction in this patient by defining the phenotype and functionality of phenindione-specific T-cells, and by using T-cell clones, we have explored drug recognition by MHC-restricted T-cell receptors.

**Materials and Methods**

**Patient Case Summary.** The patient, a 46-year-old woman, developed a deep venous thrombosis and was prescribed warfarin. However, she did not respond to warfarin and was therefore changed to phenindione, with which she managed to become therapeutically anticoagulated. Thirty days after starting phenindione, the patient developed a maculopapular rash on her legs. This was not initially suspected to be due to phenindione, which was continued until the patient was admitted to the hospital 13 days later, at which time her symptoms had progressed. At the time of hospital admission, the patient was found to have a generalized maculopapular erythematous rash associated with a temperature of 39°C, generalized lymphadenopathy, mild jaundice (peak bilirubin 35 µM, normal range <17), and bilateral patchy consolidation on the chest X-ray. Her eosinophil count was elevated at 2.5 × 10⁹/l (normal range 0–4.0), whereas alanine aminotransferase level peaked at 1115 U/l (normal range <35). On admission, phenindione was stopped, although the diagnosis was unclear at that time. Nondrug-induced causes of persistent eosinophilia and pulmonary symptoms were excluded by investigations (including stools for ova and parasites, viral and parasitic serology, autoantibodies, skin biopsy, liver ultrasound, computed tomography scan of lungs, and pulmonary function tests). A sepsis screen, including repeated blood cultures, was found to be negative. The patient did not have a history of atopy. On the basis of the above, a clinical diagnosis of phenindione hypersensitivity was made. The patient was also treated with prednisolone, after which she improved slowly over the next 6 weeks, during which time the steroids were gradually withdrawn.

Blood was obtained from the patient 3 weeks after complete resolution of her symptoms and 6 weeks after stopping prednisolone. Blood was also obtained from four phenindione-exposed nonallergic controls. Lymphocytes were obtained by centrifugation of blood layered onto Lymphoprep. Approval for the study was obtained from the Liverpool local research ethics committee and informed consent was obtained from each participant.

**Culture Medium and Chemicals.** Culture medium consisted of RPMI 1640 supplemented with 10% pooled heat-inactivated human AB serum, HEPES buffer (25 mM), L-glutamine (2 mM), transferrin (25 µg ml⁻¹), streptomycin (100 µg ml⁻¹), and penicillin (100 U ml⁻¹). The above reagents were obtained from Sigma Chemical (Poole, Dorset, UK). For culture of the T-cell clones, the media were enriched with human recombinant IL-2 (60 U ml⁻¹; PeproTech EC Ltd, London, UK); an Epstein-Barr virus-transformed B-lymphoblastoid cell line, for use as an autologous population of antigen presenting cells, was generated from the peripheral blood of the drug-allergic donor by transformation of B-cells with supernatant from the Epstein-Barr virus-producing cell line B9–58 (obtained from Dr. D. Neumann-Haefelin, University of Freiburg, Freiburg, Germany). Transformed cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, HEPES buffer (25 mM), and L-glutamine (2 mM).

Phenindione, indan, indanone, 1,3-indandione, 2-phenylindene, 2-phenylindole, 3-iminoisoolindoline, and S- and R-enantiomers of warfarin were obtained from Sigma Chemical. For T-cell culture, drugs were used at concentrations that did not inhibit the T-cell proliferative response to the mitogen phytohemagglutinin (1 µg ml⁻¹). Stock solutions (10 mg ml⁻¹) were prepared in a mixture of culture media and dimethyl sulfoxide (4:1 v/v) and diluted before use. All general reagents were purchased from Sigma Chemical and were of the best available grade.

**Lymphocyte Proliferation with Phenindione.** Proliferation of lymphocytes from the allergic patient and controls was measured using the lymphocyte transformation test, as described previously (Nyfeler and Pichler, 1997). Briefly, freshly isolated lymphocytes (1.5 × 10⁶; total volume 0.2 ml) were incubated with phenindione (1–500 µg ml⁻¹) or tetanus toxoid (0.1 µg ml⁻¹; positive control) in 96-well U-bottomed tissue culture plates for 6 days (37°C; 5% CO₂). Proliferation was determined by the addition of [³H]thymidine (0.5 µCi) for the final 16 h of the incubation period. Proliferative responses were calculated as stimulation indices (SI; cpm in drug-treated cultures/cpm in cultures with dimethyl sulfoxide alone).

**Generation and Characterization of Phenindione-Specific T-Cell Clones.** Lymphocytes (2 × 10⁶; total volume 1 ml) from the phenindione allergic patient were incubated with phenindione (10–50 µg ml⁻¹) on day 6 and 9, IL-2 was added to maintain antigen-specific proliferation. After 14 days, T-cells were cloned by serial dilution as described previously (Schnyder et al., 1997). To test the specificity of the clones (28 days after serial dilution), T-cells (0.5 × 10⁶; total volume 0.2 ml) were incubated with autologous irradiated (60 Gy) antigen presenting cells (0.1 × 10⁶) and phenindione (10 and 50 µg ml⁻¹). After 48 h, [³H]thymidine was added, and proliferation was measured by scintillation counting as described above. T-cell clones with a SI of greater than or equal to 2.5 were restimulated and expanded in IL-2 containing medium.

Phenindione-specific T-cell clones were characterized in terms of CD phenotype and T-cell receptor Vβ expression by flow cytometry. MHC restriction was determined by the addition of specific anti-HLA blocking antibodies [anti-class 1, anti-DR, anti-DP, and anti-DQ (all obtained from Dr. E. Padovan, University of Basel, Switzerland)] to the proliferation assay at concentrations known to inhibit MHC-restricted stimulations of T-cell clones. To exclude self-presentation by HLA-positive T-cells or presentation in the absence of additional antigen presenting cells, certain incubations contained T-cells and phenindione in the absence of antigen presenting cells. The functionality of the T-cell clones was investigated by measurement of phenindione-specific (1–500 µg ml⁻¹) proliferation by thymidine incorporation and cytokine secretion by ELISA. Cell cultures containing T-cells and antigen presenting cells in the absence of phenindione were taken as a control. The following ELISA kits were used: IL-4, IL-10, IFN-γ (Diaclone, Besancon, France), and IL-5 (PharMingen, San Diego, CA). The detection limits were 1 pg ml⁻¹ for IL-4, 8 pg ml⁻¹ for IL-5, and 13 pg ml⁻¹ for IL-10 and IFN-γ.

**Determination of the Specificity of the Interaction of Phenindione with MHC and the T-Cell Receptor.** To investigate the fine specificity of the interaction between phenindione and the T-cell receptor, T-cell clones were incubated with antigen presenting cells and phenindione (50–250 µg ml⁻¹) or six structurally related compounds (all 50–250 µg ml⁻¹). Chemicals were selected based on a structure disconnection approach, removing the major structural motifs of phenindione, namely the benzene ring (1,3-indandione), the keto group (2-phenylindene), or both the benzene ring and ketone groups (indan). In addition, R- and S-enantiomers of warfarin (50–250 µg ml⁻¹) were added to the proliferation assay. Proliferation was
Y

Fig. 1. Phenindione-specific lymphocyte proliferation in vitro. Results are expressed as the mean of triplicate incubations. Statistical analysis was performed by comparing incubations in the presence of drug with those in the absence of drug (*, P < 0.05). The coefficient of variation was consistently less than 20%.

Determined after 48 h by assessment of [3H]thymidine incorporation, as described above.

Generation of Three-Dimensional Energy Minimized Molecular Models of Phenindione and Warfarin. Three-dimensional structure building and molecular modeling studies were performed using SYBYL 6.91 (Tripos, St. Louis, MO) installed on a PC workstation with Red Hat Linux 7.3 operating system (http://www.redhat.com). Atomic forces were calculated by the method of Gasteiger and Marsili (1981). Energy minimizations were performed using Tripos force field with the default force field settings and charges with distance-dependent dielectric and conjugate gradient method with a convergence criterion of 0.01 kcal mol⁻¹ (Clark et al., 1989). The molecules were overlaid by performing a least-squares fit between pairs of atoms of two molecules. Phenindione was used as the reference, and the two enantiomers of warfarin were translated so that the centroid of the atoms to be fitted was superimposed on the centroid of the corresponding atoms in phenindione. The warfarin enantiomers were then rotated about this point to obtain the best possible fit between the specified pairs of atoms (Nyburg, 1974). The atoms used to match the structures are shown by asterisks in Fig. 4.

Determination of the Mechanism of Phenindione Presentation to T-Cells. The role of processing in phenindione presentation was determined using previously described methodology (Schneider et al., 1997; Zanni et al., 1998): first, by chemical fixation of antigen presenting cells and second, by measurement of the kinetics of T-cell receptor internalization, which is an early parameter of antigen presenting cells and second, by measurement of the kinetics of T-cell receptor engagement. Antigen presenting cells (2.5 × 10⁶) were fixed by the addition of glutaraldehyde (2%; 12.5 ml) in HEPES-buffered saline for 30 s at room temperature. To determine the kinetics of T-cell receptor internalization, T-cell clones (5 × 10⁴; total volume 0.2 ml) were incubated with phenindione (10 and 50 µg ml⁻¹) in 96-well U-bottomed tissue culture plates. After 1, 4, and 16 h, cells were harvested, washed, and stained for 30 min at 4°C with a fluorescein isothiocyanate-labeled anti-CD3 mAb (Serotec Ltd., Oxford, UK). CD3 fluorescence was measured by flow cytometry (Coulter Epics, XL software; Beckman Coulter, Buckinghamshire, UK). Values (100%) represent the CD3 fluorescence of T-cells incubated with antigen presenting cells in the absence of phenindione.

Statistical Analysis. Values to be compared were initially analyzed for non-normality by the Shapiro-Wilk test. Since the data were found to be non-normally distributed, we used the Mann-Whitney test for comparison of the two groups, accepting P < 0.05 as significant.

Results

Lymphocyte Proliferation with Phenindione. Lymphocytes from the phenindione allergic patient proliferated vigorously in vitro in the presence of phenindione and the positive control tetanus toxoid (Fig. 1). Concentration-dependent proliferation was observed at 10 to 500 µg ml⁻¹ phenindione (maximum SI, 141.6; 25 µg ml⁻¹ phenindione). Lymphocytes from phenindione-exposed nonallergic control patients proliferated with tetanus toxoid (SI, 15.1–38.4; 0.1 µg ml⁻¹ tetanus toxoid), but not with phenindione (maximum SI did not exceed 1.5).

Phenotype and Functionality of Phenindione-Specific T-Cell Clones. In preliminary experiments, phenindione (10 and 50 µg ml⁻¹)-specific proliferation of over 600 serially diluted T-cell cultures generated from the phenindione allergic patient was measured by the addition of [3H]thymidine. Forty-three cultures proliferated in the presence of phenindione [mean SI 5.0 ± 5.4 (10 µg ml⁻¹), 10.5 ± 6.1 (50 µg ml⁻¹); cpm 407.5 ± 253 (control); P < 0.0001 at both concentrations]; 12 were randomly selected for further analysis. Monoclonality was assessed by flow cytometric determination of T-cell receptor Vβ expression. All cultures expressed a single but differing T-cell receptor Vβ chain and are therefore, from this point on, referred to as clones (Table 1). All of the T-cell clones were CD4⁺.

Proliferation of phenindione-specific T-cell clones was concentration-dependent; maximal proliferation was observed with 25–100 µg ml⁻¹ phenindione. Concentrations of 250 µg ml⁻¹ and above inhibited proliferation. No significant proliferation was seen when T-cell clones were incubated with phenindione in the absence of antigen presenting cells. Blocking experiments with antibodies against HLA-DR, HLA-DQ, HLA-DP, and HLA-class I showed that phenindione was presented on

TABLE 1

<table>
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<tr>
<th>Clone ID</th>
<th>HLA Restriction</th>
<th>Phenotype CD and Vβ</th>
<th>Proliferation (cpm)</th>
<th>Cytokine Secretion*</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-10</th>
<th>IFN-γ</th>
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<tr>
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<td>ND</td>
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</tbody>
</table>

* Cytokine secretion measured by ELISA. Cytokine secretion expressed as picograms per milliliter.

NP, not performed due to lack of cells; ND, not detectable.

Phenindione concentrations expressed as micrograms per milliliter.

Coefficient of variation consistently less than 20%.

\[ \text{IL-4} \]
HLA-DR (5:7 clones) or HLA-DQ (2:7 clones). MHC restriction was noted when the proliferative response in the presence of blocking antibody was less than 80% of that seen with drug alone. Analysis of cytokines secreted (IL-4, IL-5, IL-10, and IFN-γ) from eight phenindione-stimulated T-cell clones revealed a mixed cytokine secretion profile. Moderate to high levels of IFN-γ (9189 ± 11001 pg ml⁻¹; range 1024–23980) was secreted by five clones, four clones secreted IL-5 (3459 ± 4009 pg ml⁻¹; range 642–9364), and one clone secreted IL-4 (4914 pg ml⁻¹). The phenotype and functionality of eight phenindione-specific T-cell clones is summarized in Table 1.

**Proliferation of T-Cell Clones with Phenindione-Related Structures and Generation of Energy Minimized Three-Dimensional Molecular Models.** Phenindione-specific T-cell clones were tested for their ability to proliferate in the presence of several structurally related chemicals (Fig. 2). Five of the seven clones were highly specific and proliferated in the presence of phenindione alone. Two clones proliferated with phenindione and weakly in the presence of 2-phenylindene; further structural modification completely inhibited the proliferative response (Fig. 2).

To study potential cross-reactivity of phenindione-specific
Phenindione was presented to six T-cell clones by fixed antigen presenting cells (Fig. 5a). However, fixation of antigen presenting cells inhibited phenindione-specific proliferation of six T-cell clones. These data indicate that phenindione is presented to T-cells via two pathways, one dependent and the other independent of processing. To confirm these data, the kinetics of T-cell receptor internalization of two T-cell clones [one potentially processing-dependent clone (clone 21) and one potentially processing-independent clone (clone 59)] was monitored by flow cytometry following phenindione stimulation. Fast (less than 1 h) and slow (4–16 h) internalization of T-cell receptors following antigen stimulation is indicative of processing-independent and -dependent drug presentation, respectively (Zanni et al., 1998). A significant down-regulation in T-cell receptor expression was observed when both T-cell clones were incubated in the presence of phenindione and antigen presenting cells (Fig. 5b). For T-cell clone 59, down-regulation in T-cell receptor expression occurred within 1 h. The extent of T-cell receptor internalization reached a plateau after 4 h. In contrast, addition of phenindione to T-cell clone 21 did not alter T-cell receptor expression between 0 and 4 h. However, significant T-cell receptor down-regulation was observed after 16 h, the time taken for antigen processing.

**Discussion**

Phenindione hypersensitivity is characterized by delayed onset of cutaneous eruptions following primary drug exposure and rapid onset following drug re-exposure (Mohamed, 1965; McMenamin et al., 1976). These features together with the detection of phenindione-specific lymphocyte proliferation in vitro (Potier et al., 1975), suggest an immune-mediated pathogenesis. Our patient developed a severe maculopapular rash and eosinophilic pneumonia after 4 weeks of treatment, which necessitated drug withdrawal. Taken together with the fact that the patient was not on any other drugs and that nondrug-induced disease was excluded, this was consistent with phenindione being the cause of her symptoms and signs.

The lymphocyte proliferation assay, which has previously been utilized to study hypersensitivity reactions to carbamazepine, lamotrigine, and sulfamethoxazole (Schnyder et al., 2000; Farrell et al., 2003; Naisbitt et al., 2003a,b), was again used in this patient to help make a diagnosis and investigate the pathogenesis. In accordance with the clinical features, lymphocytes from the phenindione hypersensitive patient, but not from phenindione-exposed nonhypersensitive controls, proliferated extremely vigorously at concentrations within the therapeutic range (6–29 μg ml⁻¹) (Schulert and Weiner, 1954). The phenindione-specific T-cells were CD4⁺, and T-cell activation required drug presentation in the context of MHC class II (both HLA DR and DQ, but not HLA DP) but not class I molecules. T-cell receptor activation also resulted in the secretion of high levels of the proinflammatory cytokine IFN-γ and moderate-to-low levels of IL-5 (from four T-cell clones) and IL-4 (from one T-cell clone), but not IL-10. The cytokine profile seen in this patient provides some insights into the immunopathogenesis of this hypersensitivity reaction. First, the secretion of IFN-γ is a common feature of T-cells from drug-induced maculopapular skin eruptions in the presence or absence of systemic symptoms (Yawalkar and Pichler, 2001; Pichler et al., 2002).
IFN-γ is known to up-regulate MHC expression on keratinocytes, rendering them more susceptible to FAS or perforin-mediated T-cell killing (Schnyder et al., 1998; Kuechler et al., 2004). Second, the secretion of IL-5 by some of the T-cell clones is consistent with the clinical manifestation of eosinophilic pneumonia in this patient. Indeed, it has been suggested that IL-5 may be involved in the pathogenesis of eosinophilic pneumonia (Allen et al., 1996). However, IL-5 is also important for cutaneous and peripheral blood eosinophilia in the absence of any lung involvement (Yawalkar et al., 2000a,b; Hashizume et al., 2002). Indeed, IL-5 production by cultured T-cells has been suggested as a possible method to diagnose drug hypersensitivity (Sachs et al., 2002). Based on the above, it can be surmised that the phenindione hypersensitivity described in this patient is an example of a type IV hypersensitivity reaction, according to the original classification proposed by Gell and Coombs (1963). This can probably be further subdivided into types IVa and IVb, according to the recently modified classification proposed by Pichler (2003).

To gain insight into the chemistry of drug recognition by T-cells, we undertook detailed cross-reactivity studies. Modification of the phenindione structure revealed that both aryl groups and the ketone contribute to the recognition of phenindione. To explore the three-dimensional aspects of drug recognition, we investigated the enantiomers of warfarin because phenindione can tautomerize to yield an enol form (Pipkin and Stella, 1982), which more closely resembles warfarin. Although some clones only recognized phenindione, three clones proliferated with phenindione, and S-warfarin and one clone proliferated in the presence of phenindione and both enantiomers of warfarin (Fig. 3). In silico energy minimized molecular models revealed that both the R- and S-enantiomers of warfarin can adopt conformers with a spatial arrangement similar to phenindione, thus providing an explanation for the cross-reactivity of certain clones with the two drugs. These data indicate that warfarin administration to phenindione allergic patients could lead to hypersensitivity. However, we are not aware of any case reports of this in the literature. Nevertheless, it is interesting to note that warfarin by itself can cause cutaneous eruptions with rapid recurrence on re-exposure, which is consistent with an immune-mediated pathogenesis (Spyropoulos et al., 2003). Whether the converse occurs, i.e., the development of cross-sensitivity with phenindione following an initial reaction to warfarin, is unclear, but may be more important in the clinical setting given the widespread use of warfarin.

Finally, we analyzed the role of antigen processing in phenindione presentation to T-cells. Glutaraldehyde-fixed antigen presenting cells present preprocessed antigens, but not antigens that require antigen processing (Zanni et al., 1998). In keeping with previous studies of drugs such as sulfamethoxazole, lidocaine, carbamazepine, and lamotrigine (Zanni et al., 1998; Schnyder et al., 2000; Naisbitt et al., 2003a,b), certain phenindione-specific T-cell clones proliferated in the presence of phenindione bound directly to MHC in the ab...

Fig. 4. In silico modeling of phenindione and warfarin. a, generation of energy minimized molecular model of phenindione in its enol form (blue) overlaid with R- (red) and S-warfarin (green) enantiomers. b, phenindione (blue) and R- (red) or S-warfarin (green) enantiomers overlaid by forcing warfarin to take up a similar three-dimensional disposition of groups as phenindione. Atoms used to match the structures of phenindione and warfarin are shown by asterisks.
sence of antigen processing (Fig. 5a), whereas others required antigen processing for T-cell receptor activation. These data were confirmed by evaluation of the kinetics of T-cell receptor internalization, an early measure of T-cell receptor activation (Fig. 5b). Thus, internalization of T-cell receptors was either rapid signifying processing-independent drug presentation (Zanni et al., 1998) or did not occur until 16 h, the time required for antigen processing of haptenated proteins (Brander et al., 1995). There are two possible scenarios that may result in processing dependent phenindione-specific T-cell activation: first, phenindione may be metabolized in vitro to a protein-reactive intermediate that binds covalently to cellular proteins. This possibility is difficult to explore since the metabolism of phenindione in liver cells in vitro and in patients has not been defined, and mass spectrometric analysis of cell culture supernatant was not sufficiently sensitive to identify a protein reactive species derived from phenindione (results not shown). Second, phenindione is unstable in light and air; major oxidation products are 3-aryl-4-hydroxyisocoumarin and the dimer 2,2'-diaryl-[2,2'-biindan]-1,1',3,3'-tetraone (De Vries et al., 1977). Additionally, phenindione is converted to a light rearrangement product 3-benzylidenephthalide, which has the potential to covalently modify lysine rich protein (Bundgaard, 1975). Thus, T-cells may be exposed to both parent drug and a covalently modified protein within the same in vitro system. The advent of proteomic technology may assist identification of the nature and origin of the modified processed peptide presented to T-cells.

In conclusion, we have characterized T-cells in a patient with phenindione hypersensitivity. Phenindione can be presented in vitro in a MHC class II restricted fashion to CD4 T-cells, via two mechanisms, one dependent and the other independent of antigen processing. T-cell receptor activation resulted in proliferation and the secretion of IFN-γ and IL-5, consistent with the involvement of both the skin and lungs in this patient. Stimulation of certain phenindione-specific T-cell clones with warfarin suggests that warfarin administration to phenindione hypersensitive patients could lead to the development of cross-sensitivity and the occurrence of a hypersensitivity reaction to warfarin.

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References


