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In vitro rapid diagnostic tests for severe drug hypersensitivity reactions in children

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ABSTRACT

Background: Previous reports have demonstrated the utility of T-cell proliferation and cytokine release assays as in vitro diagnostic tests for drug causation in drug hypersensitivity reactions (DHR). However, data from pediatric populations are scarce compared with data in adults.
Objectives: To compare the lymphocyte proliferation assay (LPA) with combination cytokine assays in the pediatric population and to identify its potential use in the acute and postrecovery phases.
Methods: A total of 18 in vitro tests were undertaken ex vivo to compare drug-specific proliferation and cytokine release (interferon-γ [IFN-γ] and interleukin-4 [IL-4]). The study included 16 patients with DHR: 7 children tested in the acute phase, 7 tested after recovery, and 2 tested during both the acute and postrecovery phases.
Results: The sensitivity of the LPA was better during the acute stage of DHR in children. Cytokine assays revealed a higher frequency of positive drug-specific responses compared with LPA in both the acute (LPA, 77.8%; IFN-γ, 88.9%; IL-4, 100%) and postrecovery phases (LPA, 33.3%; IFN-γ, 66.7%; IL-4, 66.7%). Combination cytokine assays (IFN-γ and IL-4) produced higher positive drug-specific responses in identifying culprit drugs compared with LPA in both the acute and postrecovery phases.
Conclusions: In vitro drug-induced T-cell proliferation and cytokine release assays are useful for identification of the causative drug in children with DHR. Cytokine assays (IFN-γ and IL-4) were better than LPA, but when combined, they offer even greater utility in the diagnosis of acute and postrecovery DHR. Cytokine detection is rapid and does not involve radioactivity. These novel in vitro assays may offer a significant advancement in our future management of DHR in children.

Introduction

Drug hypersensitivity reactions (DHR) are common and important concerns for health care professionals, especially in the pediatric setting.[1] There is an overdiagnosis of DHR in children with a parent-reported prevalence of approximately 10%, and these diagnoses are 1.5 times less likely to be confirmed compared with adult diagnoses.[2] Although IgE-mediated drug allergy is usually easily recognized and treated, delayed-type (T-cell–mediated) hypersensitivity is often hard to diagnose.[3] Such T-cell–mediated reactions may range from mild to severe or life-threatening and include mild maculopapular exanthem (MPE), drug reaction with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson syndrome or toxic epidermal necrolysis (SJS/TEN).[3] These conditions may cause severe life-threatening reactions or discomfort, parental anxiety, and recurrent visits to health care professionals.[4] In children, skin manifestations are often the most common presentation of these potentially severe systemic reactions.[3]

The principles of the diagnostic workup for DHR in children and adults are similar because the immunologic mechanisms involved are similar.[5] However, in reality, protocols for the clinical investigation of drug allergy are usually different in adults and children.[5] Intradermal tests are painful, whereas oral provocation tests can pose practical difficulties and are poorly tolerated in children.[5] Furthermore, these in vivo tests must only be undertaken after the clinical problem has resolved and, for delayed-type hypersensitivity, can carry a risk of untreatable life-threatening reactions.[1] Therefore, the use of in vitro tests to identify drug-specific T cells in peripheral blood of patients with DHR-induced skin eruptions may have specific advantages in the pediatric population.

We previously described a cohort of 43 tested individuals, which included 9 pediatric cases.[6] Despite the small numbers,

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the pediatric cases had similar results to the rest of the cohort. However, many consensus statements have highlighted the significant lack of published data specifically addressing in vitro diagnostics in the management of DHR in children.[7,8] We therefore set out to extend the case number to report the analysis of our tested pediatric population. We aimed to compare the use of the LPA vs IFN-γ and IL-4 drug enzyme-linked immunosorbent spot (ELISpot) assays in our cohort of tested pediatric patients. We also addressed the role of the assays in the acute and postrecovery phases when tested against different types of cutaneous DHR.

Methods

Patients

In this study, we retrospectively reviewed a cohort of pediatric patients who underwent testing for DHR at the Department of Dermatology, University Hospital Southampton National Health Service Foundation Trust. Children (aged 0–18 years) were diagnosed as having DHR on clinical grounds by consultant dermatologists or pediatric allergists experienced in the recognition of these reactions. After a detailed analysis of the medications ingested and the course of therapy initiation, up to 5 drugs were identified as possible culprits by the physician to be discontinued, and the patient was tested to these drugs using in vitro assays. All cases resolved on cessation of the possible culprit drugs, confirming DHR. A total of 18 in vitro tests (18 LPA, 18 IFN-γ, and 13 IL-4 tests) were undertaken in children with DHR, including 7 children tested only in the acute phase, 7 children only in the postrecovery phase, and 2 children during both the acute and postrecovery stages. Clinical reaction patterns were characterized in all cases (Table 1): MPE (n = 7), DRESS (n = 5), or SJS/TEN (n = 4). All children with SJS/TEN had confirmatory histologic findings. All acute cases and those with previous DHR had the classic skin rashes associated with T-cell–mediated hypersensitivity reactions. Acute DHR testing was defined as testing within 0 to 30 days from rash onset. Postrecovery in vitro testing was undertaken 84 to 1,145 days from the rash onset. All testing was undertaken on fresh (not frozen) samples isolated from peripheral blood.

Table 1
Child Characteristics

Patient No.	Age, y	Sex	Time From Rash to Test, d	Acute or Postrecovery Phase	Clinical Phenotype	No. of Drugs Tested
1[a]	15	F	2	Acute	MPE	5
1[b]	16	F	480	Postrecovery	MPE	4
2	2	M	14	Acute	MPE	4
3	15	M	4	Acute	MPE	2
4[a]	13	M	3	Acute	MPE	5
4[b]	13	M	98	Postrecovery	MPE	5
5	13	F	5	Acute	MPE	2
6	16	M	6	Acute	DRESS	4
7	8	M	84	Postrecovery	MPE	3
8	13	F	17	Acute	DRESS	2
9	12	F	12	Acute	SJS/TEN	4
10	13	M	255	Postrecovery	SJS	2
11	15	M	574	Postrecovery	SJS	2
12	18	M	158	Postrecovery	DRESS	3
13	9	M	185	Postrecovery	SJS	2
14	9	M	1145	Postrecovery	DRESS	4
15	3	F	16	Acute	MPE	3
16	6	F	132	Postrecovery	DRESS	2

Abbreviations: DRESS, drug reaction with eosinophilia and systemic symptoms; MPE, maculopapular exanthem; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.
[a]Same child tested in the acute phase.
[b]Same child tested in the postrecovery phase.

Enzyme-Linked Immunosorbent Spot (ELISpot) Cytokine Detection Assay

The ELISpot assays were undertaken as described previously.[6] Briefly, ex vivo peripheral blood mononuclear cells (PBMC) were isolated from whole blood and tested at 2.5×10^5 cells per well in RPMI 1640 supplemented with 100 IU/mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (Gibco, Paisley, United Kingdom), 1% sodium pyruvate (Gibco), and 10% heat inactivated human serum (Sigma, Poole, United Kingdom). PBMC were incubated with medium (negative control), staphylococcal enterotoxins B (positive control), or drug in a series of concentrations based on reported physiologic plasma concentrations. The plates were incubated overnight at 37°C in 5% carbon dioxide and were developed with streptavidin-alkaline phosphatase (Mabtech, Nacka Strand, Sweden) and alkaline phosphatase conjugate substrate kit (Invitrogen, Abingdon, United Kingdom). Spot-forming units per million cells from test and control wells were enumerated using an automated ELISpot reader (Autoimmun Diagnostika GmbH, Strassberg, Germany). Positive responses were recorded as those responses greater than the mean of all the background samples plus 2× the standard deviation (SD) of the background. Triplicate averaged test maximal values from the dose series were used for comparisons.[6] An example of the IFN-γ ELISpot results from a child with positive responses to a culprit drug (Teicoplanin) is shown in Figure 1.

Lymphocyte Proliferation Assay (LPA)

The LPA was undertaken as described previously.[6,9] Briefly, PBMC (2.5×10^6 mL^{-1}) were co-incubated with a dose series of the relevant drug (as above). Negative (medium with drug vehicle) and positive (staphylococcal enterotoxins B) controls were used in all assays. ^{3}H-thymidine was added on day 5 and the cells harvested 6 hours later for scintillation counting. The stimulation index was calculated as the fold difference between counts per minute recorded in wells stimulated by drug over the negative control. A stimulation index greater than 2 was considered a positive result.[6]

Statistical Analysis

As appropriate for non-normally distributed data, nonparametric analyses were used throughout the study (Mann-Whitney *U* test; GraphPad Prism Software, La Jolla, California). Median and interquartile range (IQR) responses are reported.

Results

A total of 16 children with DHR were investigated. The mean (SD) age of children in our study was 11.6 (±4.5) years (median, 13 years). Nine (56.3%) were male, and 7 (43.8%) were female (Table 1). All cases (n = 16) were tested to multiple drugs (38.9% of patients to 2 drugs, 16.7% of patients to 3 drugs, 27.8% of patients to 4 drugs, and 16.7% of patients to 5 drugs) (Table 1). Of the 59 drugs tested, antibiotics were most likely to cause DHR (35 [59.3%]), followed by anticonvulsants (11 [18.6%]) and antifungals (5 [8.5%]). Overall, IL-4 testing identified the culprit drug most frequently (11/13 [84.6%]) compared with IFN-γ (14/18 [77.8%]) and LPA (10/18 [55.6%]) testing. Combination IFN-γ and IL-4 testing identified a culprit drug in 12 of 13 cases (92.3%) (Fig 2). Only 2 children tested positive to more than one possible culprit drug when combining the LPA with cytokine (IFN-γ or IL-4) detection assay.

To assess the utility of in vitro assays during the acute phase of DHR, we evaluated the test outcome in 9 patients with DHR within 30 days from rash onset (median, 6 days; IQR, 4–10 years) (Table 1). In line with previous reports, the median circulating frequency of drug-specific T cells in acute cases identified by ELISpot was 0.39×10^{-4} for IFN-γ (IQR, $0.26–2.14 \times 10^{-4}$) and 0.47×10^{-4} for IL-4 (IQR, $0.17–2.15 \times 10^{-4}$).[6] Causative drugs as identified by positive assays