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Development of a Combined Irritancy/Phenotypic Analysis Assay for the Identification and Differentiation of Chemicals With the Potential to Elicit Irritation, IgE-Mediated, or T Cell Mediated Hypersensitivity Responses

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INTRODUCTION

Allergic and irritant dermatitis account for 15–20% of reported occupational diseases, resulting in an estimated annual loss of one billion dollars due to decreased productivity [NIOSH, 1996]. There remains a need to develop rapid, reproducible, cost effective methods to screen chemicals for their ability to induce these responses. To this end several murine models have been developed. The irritancy assay, local lymph node assay, and serum total IgE-ELISA represent three models proposed to identify chemical irritants, chemical sensitizers and IgE-mediated hypersensitivity-inducing chemicals, respectively [Kimber et al., 1994; Potter et al., 1995; Woolhiser et al., 1998].

Using flow cytometric analysis, Katona et al. [1983] observed an increased level of IgE+ draining mesenteric lymph node cells in mice undergoing an IgE response to parasitic infection with *Nippostrongylus brasiliensis*. Previous studies in this lab (data not shown) and others [Sikorski et al., 1996; Gerberick et al., 1997] have shown an elevation in the percent of B220+draining lymph node cells following dermal exposure to T cell mediated hypersensitivity-inducing allergens. Using three end points: the percent increase in ear swelling, the percent increase in IgE+B220+draining lymph node cells, and the percent increase in B220+draining lymph node cells, these studies investigate the potential use of a single combined murine irritancy/phenotypic analysis assay to identify and differentiate chemicals with the capacity to elicit irritation, IgE-mediated, or T cell mediated hypersensitivity responses.

METHODS

Study Design

Female B6C3F1 mice (5–10 animals per group unless otherwise indicated) were dermally exposed on the ear pinna for 4 consecutive days by using the dosing method and ear measuring technique described by Woolhiser et al. [1998]. Irritancy was assessed 24 hr following the final chemical exposure. Eight, ten or twelve days following initial exposure, animals were euthanized, blood was drawn by cardiac puncture for IgE determinations, and the draining cervical lymph nodes were surgically removed for phenotypic analysis.

Chemicals

Tetradecane (Tet), toluene diisocyanate (TDI), dinitrofluorobenzene (DNFB), sodium lauryl sulfate (SLS), benzalkonium chloride (BkCl), and the acetone vehicle were purchased from Sigma. Trimellitic anhydride (TMA) was purchased from Aldrich Chemical. Ethanol (EtOH), the SLS vehicle, was purchased from Fisher Scientific and

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diluted to 30% with deionized water. For the initial development of this method, sensitizing doses of TDI (2.5%) and DNFB (0.15%) and an irritating concentration of SLS (40%) were employed based on results determined via local lymph node assay (TDI, DNFB) and mouse ear swelling irritancy assay (SLS) [Woolhiser et al., 1998].

Phenotyping and Antibodies

The method was previously described [Manetz et al., 1999]. Briefly, lymph nodes in PBS were dissociated using the frosted edges of two microscope slides, and approximately 1×10^6 cells per sample were stained for analysis. The cells were washed, incubated with Fc Block®, and then incubated with the appropriate fluorescent labeled antibodies for approximately 45 min at 4°C. After washing, the cells were incubated with propidium iodide (PI) solution, washed, and then resuspended in staining buffer for analysis on a Becton Dickinson FACScan or FACVantage flow cytometer. Fifteen thousand events were collected for each sample and analyzed for the percent of viable lymphocytes by using the Cell Quest 3.1f software for the Macintosh. All antibodies were purchased from Pharmingen.

ELISA

Quantification of serum immunoglobulin levels was performed by total IgE ELISA as previously described [Manetz et al., 1999]. The ELISA was performed using Dynatech Immulon-2 microtiter plates and read on a Molecular Dynamics Emax model plate reader. The hybridomas used to generate the anti-IgE antibodies and the IgE standard for the ELISA were kindly donated by Dr. Daniel Conrad, Medical College of Virginia/Virginia Commonwealth University, and purified using an abbreviated method of Liu et al. [1980].

RESULTS

An initial time course study was carried out to examine the percent of IgE+B220+ lymph node cells 8, 10, and 12 days following initial exposure. At the optimum time point for analysis, determined to be day 10 following initial chemical exposure (Fig. 1A), the level of draining lymph node IgE+B220+ cells was approximately 52 and 23 fold higher for the TDI (41%) and DNFB (18%) exposed animals as compared to the acetone control (0.8%). There was no change in the SLS (2.2%) exposed animals when compared to the ethanol control (2.5%). The total serum IgE levels of TDI exposed animals also peaked 10 days following initial chemical exposure and were found to positively correlate with the increase in IgE+B220+ cells. The total serum IgE levels for the DNFB and SLS exposed, however, were unchanged compared to the controls (Fig. 1B).

To examine the dose–response kinetics associated with the development of the IgE+B220+ phenotype, individual animals were exposed to TDI concentrations ranging from 0.1% to 5.0%. A dose responsive increase in the percentage of IgE+ B cells with values ranging from 14% to 38% was observed (data not shown). The total serum IgE levels of these animals also increased dose responsively with values ranging from 184 ng/ml to 2600 ng/ml compared to the control value of 75 ng/ml.

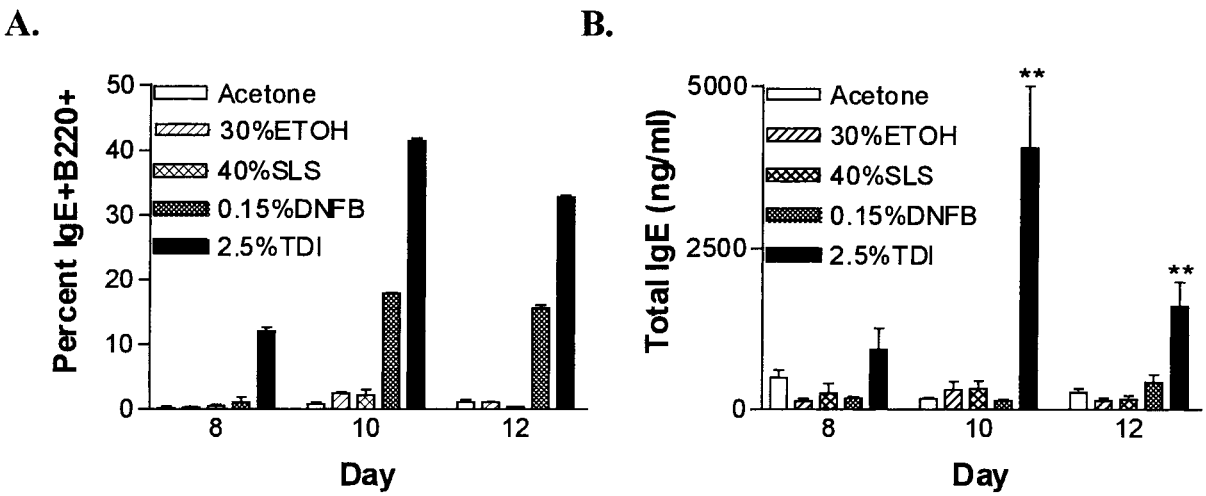

FIGURE 1. Time course of (A) IgE+B220+ cells and (B) total serum IgE levels. On days eight and ten, there were four animals per dose group except there were eight animals for the acetone and ETOH groups. At day ten, there were ten animals per dose group except for the acetone group there were 20 animals. The percent of IgE+B220+ cells is the average of three replicate samples taken from the pooled lymph nodes of each dose group at each time point. Total IgE levels are the average of individual animal values, with the number of samples ranging from two to nineteen per dose group. **represents a $P < 0.01$ for samples compared to the acetone control using the Tukey-Kramer test.

TABLE I. The Initial Paradigm and Supporting Data for a Combined Irritancy/Phenotypic Assay

Chemical	Ear swelling[a]	% B220+	% IgE+B220+[b]
Acetone	(3.6 ± 1.3)	20 ± 1.0	3.8 ± 2.0
Irritant	+	−	−
30% Tetradecane	110 ± 15**	21 ±1.5	2.7 ± 1.4
Tcell mediated	+/−	+	+/−
BkCl	40 ± 5.7**	35 ± 1.8	4.5 ± 2.3
IgE mediated	+/−	+	++++
TMA	36 ± 8.2**	41 ±1.3	33 ± 3.4**

Values in parentheses represent negative values.
[a]**represents a $P < 0.01$ compared to the appropriate control using the Tukey–Kramer test.
[b]**represents a $P < 0.01$ compared to the appropriate control using the Dunnett's t-test.

In order to differentiate irritants from sensitizers, phenotypic analysis was performed in conjunction with an existing mouse ear swelling irritancy assay. Three end points: the percent ear swelling, the percent of IgE+B220+ draining lymph node cells, and the percent of B220+ draining lymph node cells were measured. The ability of this method to identify and differentiate chemicals with the capacity to elicit irritation, IgE-mediated, or T cell mediated hypersensitivity responses was tested by using a panel of well characterized compounds. Based upon the results of these initial studies (representative chemicals shown in Table I), the following paradigm was developed for further testing.

When compared to control (acetone), irritant exposure (example, 30% Tet) resulted in an increase in percent ear swelling, with the percentage of B220+ and IgE+B220+ cells remaining unchanged. For sensitizers, exposure resulted in an increased percent of B220+ cells and depending on the irritation capacity of the compound did or did not result in an increased percent ear swelling. Exposure to a sensitizing concentration of a chemical capable of inducing IgE-mediated hypersensitivity responses (example, 50% TMA) resulted in a dramatic increase in the percent of IgE+B220+ cells, while exposure to a sensitizing concentration of a chemical that primarily induces T cell mediated hypersensitivity responses (1.0% BkCl) resulted in a moderate or no increase in the percent of IgE+B220+ cells.

In conclusion, preliminary data suggest that a combined irritancy/phenotypic analysis method by using three indicators: percent ear swelling, percent B220+, and percent IgE+B220+ cells, may allow for the identification and differentiation of chemicals with the capacity to induce irritation, IgE-mediated, or T cell mediated hypersensitivity responses.

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