The guinea pig model of diisocyanate sensitization

II. Physiologic studies

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The qualitative and quantitative properties of diisocyanate-specific IgE antisera derived from an IgE-susceptible strain of guinea pigs were investigated by passive sensitization of immunologically virgin recipient animals. These animals received infusions of antisera 48 hr before intravenous challenge with specific ligands conjugated to an unrelated carrier protein, human transferrin. The pulmonary response was sublethal and could be measured by physiologic methods. Various measurements were obtained in anesthetized guinea pigs placed in a whole-body pressure plethysmograph. Tidal volume (V_t), dynamic compliance ($C_{L\ dyn}$), and pulmonary conductance (G_L) were calculated from simultaneous recordings of transpulmonary pressure, V_t, and flow. Frequency at 1 and 2 min after intravenous challenge were also measured. Mean respiratory frequency was significantly increased in experimental animals 1 min after challenge with homologous hapten-protein conjugates, while maximum changes of V_t, $C_{L\ dyn}$, and G_L occurred 2 min after intravenous challenges. A preponderant number of physiologic abnormalities occurred in animals challenged with homologous hapten-protein conjugates. The mean percent change of V_t and $C_{L\ dyn}$ from baseline levels was significantly decreased after intravenous challenges with homologous ligands in animals passively sensitized with both hexamethylene diisocyanate– (HDI) and toluene diisocyanate–specific antisera. However, decrease of mean G_L after challenge with homologous hapten-protein conjugates was significant only in animals passively sensitized with HDI-specific antisera. When the number of positive postchallenge pulmonary function responses was compared with the mean geometric titers of IgE antisera, it was observed that moderate or severe physiologic responses were associated with infusions of IgE antisera having titers of 160 or greater. These experiments suggest that antibody concentration may be a critical determinant in distinguishing between clinical, subclinical and nonreactive states induced by diisocyanate sensitization. (J ALLERGY CLIN IMMUNOL 70:393, 1982.)

Bronchial asthma associated with inhalation of the volatile products of TDI has been recognized for many years among TDI workers.[1] The symptomatic cascade occurring after exposure to diisocyanates is variable: immediate, delayed (1 hr or more), or a combination of both.[2] Although specific IgE antibodies directed against monofunctional (*p*-tolyl or hexyl) isocyanates have been demonstrated in a small number of affected workers,[3] the precise role of immunologic mechanisms eliciting the clinical expression of symptoms is presently uncertain.[4, 5] The only reliable test to confirm TDI-mediated asthma is an inhalation challenge, which must be conducted under carefully controlled conditions.[6–9]

Since the chief clinical features of this syndrome were consistent with a possible hypersensitivity pathogenesis, early approaches to development of animal models sought to determine whether adaptive immune responses could be induced by isocyanates. Although the first investigation of this type demon-

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This work was supported in part by grant OH 733-01 from the National Institute of Occupational Safety and Health and grant ES 00159 from the National Institute of Environmental Health Sciences.

Received for publication March 5, 1982.

Accepted for publication June 11, 1982.

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0091-6749/82/110393+06$00.60/0

Abbreviations used

TDI:	Toluene diisocyanate
HDI:	Hexamethylene diisocyanate
HSA:	Human serum albumin
PCA:	Passive cutaneous anaphylaxis
TR:	Human transferrin
f_1:	Respiratory frequency at 1 min after challenge
f_2:	Respiratory frequency at 2 min after challenge
V_t:	Tidal volume
$C_{L\ dyn}$:	Dynamic compliance
G_L:	Pulmonary conductance

strated that animals immunized parenterally with TDI-protein conjugates were able to synthesize circulating antibodies, hapten specificity was not clearly established.[10] Recently, two independent laboratories obtained more clear-cut evidence of immune reactivity to isocyanates in several species of animals. One group of investigators focused attention on precipitating and homocytotropic antibodies induced by monofunctional (*p*-tolyl and hexyl) isocyanates in the guinea pig.[11, 12] Our laboratory concentrated its efforts on chemically stable protein conjugates of bifunctional diisocyanate compounds, which were found to induce the synthesis of hapten-specific homocytotropic antibodies in both mice and guinea pigs immunized with these reagents.[13, 14] Both IgG_1 and IgE types of homocytotropic antibody were demonstrated in different strains of guinea pigs. The purpose of the current investigation was to determine the qualitative and quantitative properties of diisocyanate-specific IgE antisera derived from an IgE-susceptible strain of guinea pigs by passive sensitization of immunologically virgin recipient animals.

MATERIALS AND METHODS

Preparation and characterization of guinea pig IgE antisera

Groups of six English short-hair strain guinea pigs were immunized intraperitoneally for IgE antibody production with 10 μg of TDI/HDI-HSA conjugates mixed with 1 mg of aluminum hydroxide (alum) every 30 days for a total of eight doses. Blood samples were drawn 7 days after the last booster injections. Serum from each guinea pig was stored individually in small aliquots at −20° C until used. Hapten specificity and relative potencies of individual guinea pig antisera were determined by PCA in Hartley strain guinea pigs over a 168 hr sensitization interval.[15] The reciprocals of mean PCA (RPCA) titers of individual TDI and HDI specific antisera are summarized in Table III.

Passive sensitization

Two groups of six Hartley strain female guinea pigs were each passively sensitized through the cephalic vein with 0.5 ml of guinea pig IgE antisera against TDI-HSA and HDI-HSA, respectively. Each experimental animal was injected with a different IgE antiserum according to a randomized schedule. Another group of six female guinea pigs was injected with control antisera produced by immunizing animals with normal saline and alum. All animals received infusions of respective antisera 48 hr before a challenge elicited by intravenous injection of specific ligands conjugated to an unrelated carrier protein, TR, and appropriate controls. There were no significant differences between the mean weights of the three groups of animals.

Physiologic measurements

In preliminary passive sensitization experiments with homologous diisocyanate IgG_1 antisera produced in Hartley strain guinea pigs, it had been determined that pulmonary responses were sublethal under the conditions of parenteral passive sensitization described above. Therefore it was possible to monitor the endpoint and degree of responsiveness by more quantitative physiologic methods.

Animals were anesthetized with urethane (500 mg/ml) at a dose of 1500 to 2500 mg/kg administered intraperitoneally. The left foreleg was shaved and a window was cut in the skin to expose the accessory cephalic vein. Saline (0.85%)-filled PE-10 tubing was threaded 1 cm into the vein through an incision and tied in place. This catheter was used for all intravenous challenge experiments.

The neck was shaved and the trachea exposed through a 2 cm longitudinal incision of the skin. A 3.8 cm long snug-fitting cannula was placed into the trachea through a transverse slit made just caudad to the cricothyroid cartilage and tied in place.

The animal was then placed supine into the animal chamber section of a whole-body pressure plethysmograph as described by Vinegar et al.[16] The open end of the tracheal cannula was connected to a copper tube protruding through a rubber stopper that sealed the animal chamber. A constant flow of fresh air was provided through a side port in the copper tube to keep the cannula dead space to a minimum. The other end of the animal chamber was connected to a larger reservoir chamber, a bottle stuffed with copper sponge, which ensured isothermal conditions during the calibration and measuring procedures. The pressure in the chamber was detected with a Validyne MP 45 transducer through a port in the animal chamber. The reference side of the transducer was connected to a second copper sponge–filled bottle, which provided a stable reference pressure isolated from the effects of local pressure transients. Both copper sponge–filled bottles were wrapped in polyurethane foam to insulate them from local temperature changes. The pressure transducer provided volume information. Volume calibration, linearity, hysteresis, and zero return were checked by injecting and withdrawing serial increments of air with a syringe while monitoring the signal on a Grass

TABLE I. Comparative analysis of pulmonary function measurements of diisocyanate-sensitized and saline-injected guinea pig recipients after intravenous challenges with saline, heterologous carrier protein (TR), or specific haptens coupled to heterologous carrier protein (TDI/HDI-TR)

Sensitizing agent	Challenge test	Pulmonary function tests (mean % of baseline ± SD)*				
		f_1	f_2	V_t	$C_{L\ dyn}$	G_L
TDI-HSA	Saline	100.9 ± 3.0	101.2 ± 2.2	105.9 ± 5.3	92.6 ± 12.5	98.0 ± 8.2
TDI-HSA	TR	103.6 ± 6.1	108.2 ± 12.0	103.0 ± 5.2	81.5 ± 23.9	93.9 ± 26.1
TDI-HSA	TDI-TR	151.8 ± 48.6	102.7 ± 55.2	80.4 ± 37.6	47.3 ± 50.6	58.5 ± 62.9
Saline	TDI-TR	101.9 ± 14.1	102.8 ± 9.5	102.7 ± 5.9	95.3 ± 11.9	93.5 ± 5.4
p value†		p = 0.006‡	p > 0.30	p = 0.115	p = 0.037‡	p = 0.19
HDI-HSA	Saline	94.0 ± 12.4	95.5 ± 14.0	108.3 ± 9.7	98.2 ± 10.7	102.3 ± 22.1
HDI-HSA	TR	95.9 ± 18.3	96.4 ± 20.7	108.0 ± 16.3	95.4 ± 18.9	93.8 ± 8.8
HDI-HSA	HDI-TR	212.4 ± 110.1	113.2 ± 48.6	78.1 ± 30.3	52.1 ± 34.8	56.7 ± 39.7
Saline	HDI-TR	103.1 ± 16.7	107.2 ± 20.5	97.2 ± 5.8	86.4 ± 10.8	93.7 ± 12.8
p value†		p = 0.004‡	p > 0.30	p = 0.029‡	p = 0.004‡	p = 0.017‡

*Baseline refers to physiologic measurements after the 15 min equilibration period and prior to saline injections.
†Probabilty of difference occurring by chance as determined by one-way analysis of variance.
‡Significant difference between means.

polygraph. The volume signal was differentiated, amplified, and displayed on a polygraph channel. The resulting flow signal was calibrated by introducing a triangular wave "volume" signal to the volume channel while adjusting the resulting square-wave "flow" signal to an appropriate level. Transpulmonary pressure was determined by measuring airway opening pressure and esophageal pressure separately and electronically subtracting the two signals. Airway opening pressure was detected with a Validyne MP 45 transducer and esophageal pressure with a Hewlett Packard 1280 transducer attached to a water-filled catheter, the open end of which was inserted into the esophagus to a level that gave maximally negative swings of pressure. Each transducer was also connected to a Hewlett Packard 8805 B carrier amplifier for signal conditioning.

Respiratory frequency was determined over 20 sec intervals after the f_1 and f_2 points. All other physiologic indices were measured 2 min after intravenous challenge because maximal changes of these parameters occurred at this time. V_t was measured from the trough to the peak of each cycle and a mean value was calculated from three successive respiratory cycles. $C_{L\ dyn}$ was calculated by dividing V_t by the difference in transpulmonary pressure between inspiration and expiration. These results were expressed as the means of three successive respiratory cycle measurements. Pulmonary resistance was determined by dividing the difference of transpulmonary pressure between inspiration and expiration by the difference in flow at mid-V_t for each of three successive breaths.[17] The results were expressed as G_L and mean values were obtained. Positive physiologic responses were defined as values exceeding 2.5 standard deviations from mean values of respective pulmonary function parameters obtained in control animals after they were challenged intravenously with saline.

Since all studies were performed in anesthetized animals, there was an expected decrease of $C_{L\ dyn}$ values during the time course of the serial challenges. However, the degree of decrease was minimal and not significantly different from the beginning to the end of the challenge protocol in the control animals (100.32 ± 6.85 vs 95.30 ± 11.85 percent of baseline, $p > 0.30$). Thus theoretical errors of $C_{L\ dyn}$ attributable to "anesthesia drift" during the time course of the experimental procedure were not detected in control animals.

Challenge protocol

On the experimental day, the animal was cannulated as described above and placed in a plethysmograph. After equilibration for 15 min a baseline recording of transpulmonary pressure, V_t, and flow was made. The animal then received successive intravenous injections through the venous catheter. The initial intravenous injection consisted of 0.5 ml of 0.85% saline. Five minutes later, animals received injections of 0.5 ml of TR (4 mg/ml). After another interval of 5 min, TDI-HSA–sensitized animals received 0.5 ml of TDI-TR and HDI-HSA–sensitized animals received 0.5 ml of HDI-TR. Control animals received TDI-TR followed 15 min later by HDI-TR, or vice versa, in a randomized fashion. Finally, animals that showed no responses to any of the challenges received a 0.5 ml dose of histamine (0.01 mg/kg) to check the degree of histamine responsiveness of the guinea pig under study. In all experiments, injection of histamine resulted in strong responses, thus confirming the patency of the preparation.

RESULTS

Changes of various pulmonary function indices occurring after challenge with saline, TR, the respective isocyanate conjugates, or histamine were related to

TABLE II. Number of positive physiologic responses* in sensitized and saline-injected animals

Animals sensitized and injected with:	Positive pulmonary function tests after challenge with:															
	Saline				TR				TDI-TR				HDI-TR			
	f_1	V_t	$C_{L\,dyn}$	G_L	f_1	V_t	$C_{L\,dyn}$	G_L	f_1	V_t	$C_{L\,dyn}$	G_L	f_1	V_t	$C_{L\,dyn}$	G_L
TDI-HSA (n = 6)	0	0	1	0	0	0	2	1	3	2	3	3	NT	NT	NT	NT
HDI-HSA (n = 6)	0	0	0	0	0	0	1	1	NT	NT	NT	NT	5	2	6	4
Saline (n = 6)	0	0	0	0	1	0	1	1	0	0	1	0	0	0	2	1

NT = not tested.

*A positive response is defined as either a decrease (V_t, $C_{L\,dyn}$) or increase (f_1, G_L) in excess of 2.5 SD from mean pulmonary function measurements obtained in control animals after intravenous injection of saline.

reference baseline values and recorded as percent of baseline in the assessment of individual animal results. Baseline physiologic status was assessed by comparing prechallenge pulmonary function measurements in both experimental and control groups. No significant differences were noted. Mean percent baseline data of experimental and control groups are displayed in Table I. Mean respiratory frequency was significantly increased in both experimental animal groups 1 min after the challenge with homologous hapten-protein conjugates. No significant respiratory rate changes were observed after saline and carrier protein (TR) challenges of experimental animals. As expected, the only challenge substance capable of altering the breathing rate response in control animals was histamine (data not shown). In contrast to the mean f_1 results, there were no significant differences of f_2 changes between experimental and control groups.

Maximum changes of V_t, $C_{L\,dyn}$, and G_L typically occurred 2 min after challenges. The mean percent change of V_t and $C_{L\,dyn}$ from baseline levels was significantly decreased after intravenous challenges with homologous ligands in both experimental groups, while the only significant change in these indices in the control group occurred after the histamine challenge. Decrease of mean G_L after challenge with homologous hapten-protein conjugates was significant only in the animals passively sensitized with HDI-specific antisera. Although three animals in the TDI passively sensitized group experienced marked decreases of G_L after challenge with the TDI conjugate, three other animals in this group did not exhibit changes of this index. Thus the mean percentage change of G_L in the TDI passively sensitized animals was not significantly below baseline values ($p = 0.19$).

Experimental results occurring in individual animals after control or specific challenges are summarized in Table II. It is evident that a preponderant number of physiologic abnormalities occurred in animals challenged with homologous hapten-protein conjugates or histamine. Although physiologic data obtained after intravenous injection of saline and TR were similar in all three animal groups, there were a few unexpected responses. One TDI-HSA–immunized animal showed marginal changes in $C_{L\,dyn}$ after saline challenge and borderline changes of $C_{L\,dyn}$ and G_L after TR challenge. However, this animal demonstrated marked changes in f_1, V_t, $C_{L\,dyn}$, and G_L after the intravenous challenge with TDI-TR. One HDI-HSA–immunized animal showed a marginal decrease of G_L after intravenous challenge with TR. Another HDI-immunized animal had a decrease in $C_{L\,dyn}$ after TR injection as significant as that it had experienced after the challenge with homologous hapten conjugate. Positive pulmonary responses occurring in the group of animals injected with saline alone could all be attributed to minor degrees of biologic variability inherent in an in vivo physiologic preparation of this type.

Comparative analysis of the number of positive postchallenge pulmonary function responses and the reciprocal mean geometric titers of IgE antisera passively infused into each animal revealed several interesting associations (Table III). With one exception (TDI animal 6), moderate or severe physiologic responses were associated with sensitizing infusions of more potent IgE antisera having mean reciprocal titers of at least 160. Lack of response or relatively minor responses were noted in animals sensitized with low-titered IgE antisera (TDI animals 4 and 5, HDI animal 4).

DISCUSSION

Pulmonary anaphylactic responses elicited by diisocyanate protein conjugates in guinea pigs passively sensitized with specific IgE antisera generally conformed to the same pattern of previous localized cutaneous anaphylaxis experiments.[14] In further corroboration of these prior results, pulmonary reactions oc-

curred only after challenge with specific ligands coupled to a heterologous protein carrier, TR. All but one animal passively sensitized with HDI-specific IgE antisera demonstrated abnormalities in one or more pulmonary function parameters after intravenous challenge with HDI-TR. Equivalent results were obtained in three of six animals sensitized with TDI-specific antisera and challenged with TDI-TR. The validity of negative reactions in three guinea pigs of the TDI-sensitized group was corroborated by demonstration of significant pulmonary responses to infused histamine at the conclusion of the challenge protocol.

It was noteworthy that mean reciprocal PCA titers of negatively responding animals were lower (Table III; RPCA titers of 7 and 57) than TDI-specific IgE antisera that evoked positive responses (Table III; RPCA titers of 160, 320, and 1860) except for TDI animal 6, which did not respond after passive sensitization with an antiserum having an RPCA titer of 226. In the group of animals passively sensitized with HDI-specific IgE antisera, correlation of the number of physiologic responses with the degree of PCA titer was more pronounced. Thus positive pulmonary responses occurred in all animals passively sensitized with IgE antisera having RPCA titers of 453 or greater. One recipient animal passively sensitized with an HDI-specific antiserum of relatively low titer (RPCA titer of 57) did not show significant pulmonary function abnormalities after intravenous challenge with HDI-TR.

All positively responding animals exhibited a significant increase in respiratory frequency within 1 min after intravenous challenge with the eliciting hapten-protein conjugate. In some instances this increase in frequency persisted until significant changes of $C_{L\ dyn}$ and G_L occurred 2 min after the initial challenge. Minimal reactions appeared to be confined to isolated increases of f_1 without concomitant changes in V_t, $C_{L\ dyn}$, and G_L. Decreases of f_2 below baseline values 2 min after challenge generally preceded or appeared at the same time as more widespread changes of V_t, $C_{L\ dyn}$, and G_L.

Previous investigators using the monofunctional isocyanate guinea pig model proposed that respiratory frequency alone could be used as a reliable objective indicator of pulmonary hypersensitivity reactions after appropriate challenge of actively or passively sensitized guinea pigs.[11] However, the rapid and variable fluctuations of respiratory rate obtained in the current experience indicate that this parameter cannot be utilized as the sole index of a physiologic response.

A greater number of physiologic abnormalities tended to occur in animals passively sensitized with higher concentrations of IgE antisera, whereas minimal or complete unresponsiveness was more likely to be associated with less potent IgE antisera. For unknown reasons, an exception to this correlation was observed in one of the TDI-sensitized animals (TDI animal 6) that did not respond after challenge with specific ligand even though it had been sensitized with a relatively high titer of specific IgE antiserum (Table III; RPCA titer of 226).

Haptenic crossreactivity could not be investigated systematically in the current experiments because

TABLE III. Profile of physiologic responses of guinea pig recipients after intravenous challenges with diisocyanate conjugates compared with mean PCA titers of IgE antibodies used for passive sensitization*

Animal challenged with:	No.	f_1	V_t	$C_{L\ dyn}$	G_L	PCA†
	TDI-HSA antisera passive immunization					
TDI-TR	1	+	+	+	+	320
	2	+	+	–	+	1860
	3	–	–	–	+	160
	4	–	–	–	–	57
	5	+	–	–	–	7
	6	–	–	–	–	226
	Saline‡					
	1	–	–	+	–	Neg
	2	–	–	–	–	Neg
	3	–	–	–	–	Neg
	4	–	–	–	–	Neg
	5	–	–	–	–	Neg
	6	–	–	–	–	Neg
	HDI-HSA antisera passive immunization					
HDI-TR	1	+	–	+	+	905
	2	+	–	+	–	453
	3	+	–	+	+	905
	4	–	–	+	–	57
	5	+	+	+	+	905
	6	+	+	+	+	453
	Saline‡					
	1	–	–	+	–	Neg
	2	–	–	–	–	Neg
	3	–	–	–	–	Neg
	4	–	–	–	–	Neg
	5	–	–	+	+	Neg
	6	–	–	–	–	Neg

*A positive (+) response is any value that exceeds 2.5 SD from the mean of values obtained in control animals after intravenous injection of saline. A non-reactive (–) response is defined as any value within 2.5 SD of the mean pulmonary function measurements obtained in control animals after injection of saline.

†Expressed as the geometric mean of reciprocal PCA titers obtained in two recipient animals.

‡Six control animals were challenged successively with TDI-TR and HDI-TR.

technical reliability would be compromised by the prolonged anesthesia that would be required for recovery from previous responses. However, it was possible to add a heterologous diisocyanate conjugate to the challenge protocol of one animal (TDI-TR animal 5) that exhibited a minimal response to the homologous hapten-protein conjugate. In this single instance there was no apparent evidence of haptenic crossreactivity between TDI and HDI.

Recent reports that monofunctional isocyanate-specific IgE antibodies occur in small subsets of workers with TDI-induced asthma indicate that hypersensitivity mechanisms may be implicated in the pathogenesis of this syndrome.[3–5] However, the fact remains that workers are exposed to bifunctional, not monofunctional, compounds in the workplace. Since it has now been clearly established that susceptible strains of guinea pigs produce hapten-specific IgE antibody after immunization with bifunctional diisocyanate conjugates, the ultimate prospect of proving IgE-mediated clinical hypersensitivity to these compounds in humans now appears to be based on firmer experimental background. Moreover, studies with the guinea pig suggest that the antibody concentration threshold may be one of the critical determinants in distinguishing between clinical, subclinical, or nonreactive states. The principles of innate susceptibility and a critical threshold of antibody concentration, which itself is dependent on extent and duration of antigen exposure, are consistent with the generally accepted observation that TDI asthma occurs only in a small subpopulation of exposed workers.

We gratefully acknowledge the technical assistance of Ms. Susan Osmanski and the secretarial assistance of Ms. Jan Shulman.

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