DIISOCYANATES PANEL

Scientific Information Statement (with references)

TDI Antibody Testing

Toluene diisocyanate (TDI) is a chemical compound that reacts rapidly with polyols and amines to form flexible polyurethane foam. Exposure to TDI at levels above exposure limits† designed to provide safe workplace environments may cause individuals to experience asthma-like symptoms upon re-exposure to TDI at levels even below the exposure limit.† Although the exact mechanism remains unclear, some cases of TDI-induced asthma have been considered to be the result of antibody formation resulting in allergic disease.† Antibody formation, as well as cell-mediated immune mechanisms, and non-immune mechanisms have been investigated.†

Background

Antibody formation is a general defense against organisms and substances that are foreign to the body. The detection of specific antibodies can be used to determine whether the immune system of an individual might have been triggered as a result of contact with a particular foreign material. The presence of antibodies, in itself, is not considered to be a definitive indication of allergic disease, such as asthma.7,8,9 Allergic disease occurs when the immune system reacts to a foreign substance and causes a response that might result in sneezing, itchy eyes, hives, or breathing difficulty. Antibody production is only one of the components of this type of response. Similar responses may also be caused by non-immune mediated irritation.

Despite considerable research around the world over the last 20 years, it is not yet possible to reliably correlate serum levels of antibodies with clinical signs of airway diseases such as TDI-related asthma.10,11,12 TDI-related asthma can be more accurately diagnosed using a stepwise approach to determine whether there is a relationship between the individual’s asthmatic symptoms and a specific triggering activity in the workplace. This includes a carefully collected work history, respiratory questionnaire, and examination of lung function. Observation to determine whether pulmonary effects occur upon exposure to low levels of TDI and abate when exposure is avoided is key to the assessment.13,14,15,16 Specific provocation challenge with TDI is considered the ‘gold standard’, an effective and highly reliable diagnostic tool, but it is not readily available and is also expensive.13,15,16,17

Among reports investigating the mechanism of TDI asthma, several researchers have reported that in vivo TDI can react with carrier proteins to form a complex,18,19 which the immune system could recognize as “foreign”.4,18 This may result in the production of two types of TDI-specific antibodies, IgE and IgG.4 To detect TDI-specific antibodies, an individual’s serum is mixed with TDI-protein conjugates, prepared in a laboratory, then tested with either a radioallergosorbent (RAST) or enzyme-linked immunosorbent (ELISA) assay. The results for specific IgE or IgG are expressed as a titer of percent bound radioactivity for the RAST assay or optical density for ELISA.11,20,21 A review of the medical literature indicates that there are a variety of methods in use to prepare the TDI-protein conjugate as well as protocols to perform the assay, and that no standard protocol has achieved general consensus. In addition, the criteria for a test to be considered positive have not been standardized and vary among laboratories.1,22,23 Therefore, the

† Material Safety Data Sheets, available from TDI suppliers, provide additional health and safety information regarding this chemical.
results from one laboratory often are not comparable to those from another laboratory. Interpretation of TDI-specific antibody assay results is, thereby, complicated and uncertain.

**Accuracy and Validity of Antibody Testing**

Current antibody tests do not reliably identify individuals with or without TDI-related asthma as the presence of antibodies does not necessarily indicate disease.\(^6,10,11\) Like most medical tests, antibody assays may yield both false negative and false positive results.\(^11\) More specifically:

1. Some investigators have suggested that individuals who have worked with diisocyanates and have diisocyanate-specific antibodies in their blood may be more likely to have an asthmatic reaction with subsequent re-exposure.\(^21,24\) However, various studies provide evidence that circulating TDI-specific IgE antibodies can be detected in 0 to 86 percent, and TDI-specific IgG antibodies in 3 to 100 percent, of individuals who showed a positive response in a specific provocation challenge (that is, showed an asthmatic reaction to a challenge test with TDI).\(^25,6,8,9,20,21,24,25,26,27\) On the other hand, TDI specific antibodies have been found in 0 to 11 percent of exposed non-asthmatic workers.\(^8,23,25,27\)

2. Researchers have tried to add various stringent criteria to produce more accurate results, but still remain challenged in predicting disease. In order to reduce the number of ‘false positive’ readings, researchers have raised the titer that is required to call a test positive \(^25,28\) and have recommended confirming questionable results with inhibition tests.\(^24,28\) Where IgE assays are conducted in a manner to ensure that false positive results are minimized, researchers state the presence of TDI-specific IgE antibodies are ‘strongly’ associated with the presence of asthma.\(^28\) However, in several studies, the IgE assay was capable of confirming the diagnosis in 0 of 4, \(^20,25\) 0 of 6, \(^24\) and 3 of 8, \(^27\) and 44% \(^29\) of individuals known to have TDI-induced asthma. Use of highly substituted TDI conjugates may increase sensitivity of the ELISA, but at the expense of unacceptable levels of binding with negative control sera and inconsistent binding with positive reference control serum.\(^30\) Results from an inter-laboratory study demonstrated a varied agreement and concordance when sharing reference sera and multiple conjugates. In this study, “false positive” TDI-specific IgG responses were seen in a small number of non-asthmatic subjects in one laboratory, when the same responses were not observed in another lab.\(^30\) Other methods of preparing conjugates, such as vapor-phase, showed a positive result in only 44% of patients with TDI asthma.\(^29\)

3. Several reports have indicated circumstances where individuals with no known exposure have shown diisocyanate-specific antibodies.\(^9,22,23,31\) A recent study found a 5% prevalence of TDI-IgG in a population of 139 individuals who have no known history of exposure to TDI.\(^31\) In addition, TDI-specific antibodies have been detected in 3-40% of controls.\(^9,22,23\) Two out of five normal controls (40 percent) without work exposure to TDI was found to show a positive test for TDI-specific antibodies and one extremely high titer of TDI-specific IgG was found in an individual with no known exposure.\(^9\)

4. A factor that may influence the predictive ability of the test is the interval from last exposure. In one study, after thirty days away from exposure, the positive antibody tests decreased from 41 percent to 14 percent.\(^25\) This relationship has not been firmly established. For example, after one year of non-exposure diisocyanate-specific antibodies were detected in 63 percent of individuals showing a positive response in a specific provocation challenge, while only 36% of recently exposed positive responders were positive for diisocyanate-specific antibodies.\(^9\)
The decrease of antibody levels usually correlates to some improvement in asthma symptoms, but may not correlate to a complete remission of the person’s asthma.\textsuperscript{32}

5. There have been many reports regarding cross-reactivity of the various diisocyanates during antibody testing.\textsuperscript{12,24,33} This cross-reactivity may be due to formation of new antigenic determinants, which may be similar to other diisocyanates.\textsuperscript{24,34} Although unproven, there may also be cross-reactivity with non-diisocyanate substances; one instance of concurrent bronchial reactivity to TDI and radishes (isothiocyanates) has been reported.\textsuperscript{34} This adds to the inaccuracy of the current antibody assays.

6. Other reasons that have been suggested for the low and variable association between detection of antibodies and presence of diisocyanate-related asthma include:

a. a small number of subjects in each study (in some studies only 1 to 2 individuals were tested)\textsuperscript{5,20};

b. different and non-validated testing methodologies;

c. variability in conjugate preparation,\textsuperscript{12,23,30} including the type of protein used; and

d. perhaps most importantly, the possibility of a non-IgE antibody-mediated immune mechanism for some cases of diisocyanate asthma.

**Summary**

In summary, given the low predictive ability and the various confounding factors presently associated with antibody testing, a positive test for TDI-specific antibodies has not proven to be a reliable, definitive marker for diagnosing TDI-related asthma. Establishing work related bronchoconstriction with serial lung function monitoring during the work week and after a period away from work is the most practical method of making the diagnosis. Specific provocation challenge with TDI is considered the ‘gold standard’, highly reliable as a diagnostic tool, but not readily available and also expensive. A body of research suggests that a positive TDI-specific IgE antibody test result may be useful in the diagnosis of TDI-related asthma only if (1) there is a strong pre-test probability of work-related asthma, (2) there is current or recent exposure to TDI, (3) the test is conducted in a manner to minimize false positive results. Research has also shown that TDI-specific antibody assays used independently have low predictive ability (confirming the diagnosis in only one out of five individuals) and have potential for inaccuracy (up to 11 percent positive responses in non-asthmatic individuals and up to 12 percent positive responses in control populations). Current test methods have not been standardized or validated. Therefore, the antibody assay does not replace any of the exposure-related physiologic measurements in making the clinical diagnosis of TDI-related asthma. Currently, the detection of TDI-specific antibodies has not demonstrated reliability as an index of adverse health effects or exposure because the current analytical method has a low degree of accuracy, or as recently proposed, diisocyanate-induced asthma is a non-IgE-mediated disease.

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References


