Hypersensitivity Reactions and Methods of Detection
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ABSTRACT
Hypersensitivity reactions are classified into four groups (Type I, II, III, and IV), each characterized by specific biological actions. Research has focused on understanding each hypersensitivity to ensure appropriate therapeutic recommendations are made. This overview will present the defining characteristics of each hypersensitivity and examine the diagnostic methods used to determine the existence of a specific type. Type I hypersensitivities can be determined by provocation testing, immediate-type skin testing, or radioallergosorbent tests (RASTs); Type II hypersensitivities can be determined by measuring the level of IgG antibodies to specific host proteins; Type III hypersensitivities may be detected with serum IgG antibody testing to specific antigens; Type IV hypersensitivities are determined by delayed skin testing or memory lymphocyte immunostimulation assay (MELISA®). Persistent antigen exposure may contribute to chronic conditions such as autoimmune diseases, irritable bowel syndrome, asthma, or even psychiatric illnesses. Through the effective detection of potential hypersensitivities, a patient’s quality of life can be improved through consequent antigen avoidance.

INTRODUCTION
A hypersensitivity reaction refers to a state of altered reactivity in which the body mounts an amplified immune response to a substance. In 1963, Gell and Coombs classified hypersensitivity reactions into four different groups (Types I, II, III, and IV) which depended upon the severity and latency of a reaction (Gell & Coombs, 1963). Clinically, it is difficult to distinguish between the four types of hypersensitivities as they do not necessarily occur in isolation from each other. Biologically, Types I, II, and III hypersensitivities are mediated by antibodies, whereas, Type IV is mediated by T cells and macrophages (Brostoff et al., 1991). Hypersensitivities can only manifest following a second or subsequent contact with a particular antigen. Recently, extensive research has been conducted to determine optimum treatments and to define the specific mechanisms that mediate each type of hypersensitivity. This overview will define the different types of hypersensitivities and examine different methods to detect hypersensitivity responses.

TYPES OF HYPERSENSITIVITY
Typically, hypersensitivities are classified into four different types. Type I hypersensitivity is an immediate immune reaction to an antigen (Sicherer & Leung, 2009). Mast cells and basophils play an integral role in Type I hypersensitivity reactions. Following exposure to an antigen, mast cells and basophils go through a process called degranulation, where they release substances that induce inflammation (Figure 1A). Specifically, antigens interact with IgE molecules that are bound to high affinity receptors on the surface of mast cells, called fragment crystallizable (Fc) receptors, to induce degranulation (Yamasaki & Saito, 2005). Mast cell degranulation can lead to the release of inflammatory mediators including histamine, proteoglycans, serine proteases, and leukotrienes (Yamasaki & Saito, 2005). The immediate release of these inflammatory mediators can produce hives, redness, and angioedema (swelling of the lips, eyelids, throat, or tongue) in what is referred to as an anaphylactic reaction (Noone & Osguthorpe, 2003). In some cases, the anaphylactic reaction can be severe enough to block airways or cause heart arrhythmias (Noone & Osguthorpe, 2003).

Type II hypersensitivities, also known as cytotoxic hypersensitivities, are rare reactions that are typically caused by IgG and IgM antibodies (Brostoff et al., 1991). A Type II response may occur when the target antigen is part of the surface of a specific host cell or tissue (Figure 1B). Type II hypersensitivities can be associated with autoimmune diseases, drug reactions, and transplantations (Brostoff et al., 1991). Biochemically, Type II reactions occur when IgG and IgM antibodies bind to host cells and tissues to form complexes that activate the complement pathway which, in turn, eliminates the host cells (Brostoff et al., 1991; Kornbrust et al., 1989). During Type II hypersensitivities, mediators of acute inflammation, such as B cells, antibodies, and cytokines are generated to induce cell lysis and death. For example, a Type II reaction may occur when a drug binds to host cells, such as red blood cells, causing them to be recognized as foreign pathogens. This will induce B cell proliferation and antibody production as well as activate the complement system which will ultimately trigger cell death (Kornbrust et al., 1989). A Type II reaction may take several hours to a full day to develop.
Type III hypersensitivities are also mediated by IgG and IgM antibodies (Table 1). Unlike a Type II response, Type III hypersensitivity is associated with responses to soluble antigens that are not combined with host tissues but with antibodies in the blood which can then lead to inflammatory responses (Brostoff et al., 1991). As the number of antigen-antibody complexes increase, they can deposit in joints and various tissues such as kidneys, skin, and eyes leading to an inflammatory response wherever they precipitate (Ellsworth et al., 2008). Research has suggested that Type III reactions may contribute to certain systemic diseases such as systemic lupus erythematosus (SLE), serum sickness, and farmer lung (Coico & Sunshine, 2009). A Type III reaction can take hours, days, or even weeks to develop.

Type IV hypersensitivities are referred to as delayed-type hypersensitivities because a reaction can typically take 12 or more hours to develop (Brostoff et al., 1991). Type IV responses are dependent on T cell interactions, which recruit other cells to the site of exposure (Brostoff et al., 1991). Upon an initial antigen exposure, naive T cells differentiate into memory T cells which are specific to that antigen. The memory T cells remain dormant until a subsequent exposure of the antigen elicits a faster and stronger immune response than the first encounter. Upon future encounters with the given antigen, memory T cells immediately proliferate and differentiate into effector cells to quickly eliminate the antigen. Contact dermatitis is commonly caused by a Type IV reaction in which lymphocyte production can produce local inflammation, such as a rash, which characterizes this skin condition (Nosbaum et al., 2009).

Recent literature proposes a fifth type of hypersensitivity in which granulomas, a ball-like collection of macrophages and epitheloid cells, are formed to encapsulate and isolate a pathogen (Rajan, 2003; Tercelj et al., 2008). Granulomas are formed in response to antigens that escape the early phases of an immune response and are often times related to a delayed-type hypersensitivity (Tercelj et al., 2008).

Although hypersensitivity reactions appear to be deleterious, they do serve the purpose to protect the human body through the isolation and elimination of specific antigens. Still, identification of hypersensitivity reactions can be beneficial for helping patients to avoid reactive antigens and to limit deleterious actions, which will decrease the risk of developing chronic diseases (Rajan, 2003).

METHODS TO IDENTIFY HYPERSENSITIVITY

Various methods exist to determine the existence and types of hypersensitivity. A summary of methods to detect Type I, II, III, and IV is provided.

Type I hypersensitivity reactions are traditionally recognized through provocation testing or immediate-type skin testing (Table 1). Provocation testing involves a masked topical challenge of certain antigens followed by observation of the patient’s dermal responses (Smith, 1992). Clinically, the provocation testing can pose a danger since some antigens may result in a severe anaphylactic reaction (Smith, 1992). Skin testing is an alternative method which may include skin pricks or patches to determine hypersensitivity. Both the prick test and scratch test involve pricking the skin with a needle or pin containing a small amount of the antigen. A patch test is conducted by applying a patch that contains known antigens to the skin (Williams et al., 1992). If there is a visual reddening or swelling at the prick or patch site, the patient is considered allergic to that antigen (Williams et al., 1992). Type I hypersensitivity reactions can also be determined via radioallergosorbent tests (RASTs), which detect the amount of IgE antibodies that react with suspected or known allergens (Primeau & Adkinson, Jr., 2001; Williams et al., 1992). If a RAST indicates a high level of IgE to a specific antigen, the person is said to be allergic to that antigen (Primeau & Adkinson, Jr., 2001). Other methods to detect a Type I hypersensitivity include leukocyte histamine release assays, surface markers for basophil activation, and leukotriene release tests (for review see Primeau & Adkinson, Jr., 2001).

Type II hypersensitivity reactions are mediated by IgM and IgG antibody responses to host tissues (Table 1). For instance, Goodpasture syndrome is an autoimmune disease characterized by
inflammation of the glomeruli in the kidneys and hemorrhaging of the lungs (Salama et al., 2001). The measurement of IgG antibodies to glomerular basement membrane (anti-GBM) is used to diagnose Goodpasture syndrome (Salama et al., 2001). In addition, anti-neutrophil cytoplasmic IgG antibodies (ANCAs) are used to detect various autoimmune disorders that may be associated with Type II hypersensitivities (Radice & Sinico, 2005). Most common Type II reactions occur in transplant and blood transfusion patients where the reaction is determined by a tissue biopsy or by monitoring a patient’s signs and symptoms.

Although limited data exists about methods for the detection of Type III hypersensitivity reactions, some suggest serum IgG antibody testing can be utilized (Table 1; Shamberger, 2008; Stapel et al., 2008). Research on food sensitivities suggests that the use of enzyme-linked immunosorbent assays (ELISA) can be effectively used to detect IgG antibodies to specific dietary proteins (Shamberger, 2008). Specifically, an increased titer of IgG antibodies to a specific dietary protein would be indicative of a Type III hypersensitivity to that protein (Scott et al., 1990). Cessation from eating the reactive food would therefore be recommended. However, further research is warranted to verify serum IgG antibody testing as a method to detect Type III hypersensitivities.

In recent years, methods have been developed and refined to detect Type IV hypersensitivities (Table 1). Historically, the role of T cells in hypersensitivity reactions has been neglected; however, recent research has clarified and analyzed the role of T cells in hypersensitivity, which has provided a better understanding of delayed-type reactions (Primeau & Adkinson, Jr., 2001). Two primary methods exist to verify a Type IV hypersensitivity in patients: 1) delayed skin testing and 2) lymphocyte transformation tests (Primeau & Adkinson, Jr., 2001).

Delayed skin testing is similar to an immediate-type skin test however the reaction is typically read after 24 or 72 hours rather than 15 minutes after application of the antigens (Li, 2002). Still, skin testing can pose the risk of developing adverse systemic reactions and appropriate training and care must therefore be practiced to ensure the safety of patients (Reid et al., 1993). In addition, research has shown that skin testing may be unreliable for some antigens, such as food allergens (Sampson & Albergo, 1984). Due to the limitations of skin testing (Rietschel, 1996), alternative methods are currently being tested for the detection of Type IV hypersensitivities including such methods as lymphocyte transformation tests (Pichler & Tilch, 2004b).

The lymphocyte transformation test is an in vitro assay that measures the proliferation of T cells following an antigen challenge (Warrington & Tse, 1979). An enhanced version of the lymphocyte transformation test, called memory lymphocyte immuno-stimulation assay (MELISA®), is available for healthcare practitioners and can assist in the detection of Type IV hypersensitivities, as previously described (Valentine-Thon et al., 2006; Stejskal et al., 1996). In summary, a standard number of lymphocytes, with the exclusion of monocytes, are isolated from whole blood specimens for cell culture. The lymphocytes are cultured for 5 days then transferred to new plates containing known antigens, which are then pulsed for 4 hours with methyl-³H-thymidine to quantify cell proliferation. A negative control is also obtained via lymphocytes from the same patient, which is not added to antigens. After culture, the lymphocytes are harvested onto filter paper and dried. The radioactivity present on the filter paper is measured in a liquid scintillation counter. A stimulation index (SI) is calculated by dividing the counts per minute (cpm) in the test well to the average cpm in the negative control wells (Valentine-Thon et al., 2007; Valentine-Thon & Schiwara, 2003; Stejskal et al., 1996). A positive reaction, indicating Type IV hypersensitivity, is defined as a SI greater than 3 and an equivocal reaction is a SI between 2 and 3. A SI less than 2 is considered negative.

Clinically, MELISA® has been proven to be an effective tool for the determination of sensitivities to various metals (Valentine-Thon & Schiwara, 2003) and to bacterial antigens such as Borrelia burgdorferi (Valentine-Thon et al., 2007). Ultimately, patients who possess hypersensitivities can intelligently choose to remove or avoid various cleaning chemicals, drugs, foods, dental amalgams, jewelry, or cosmetics that contain the hypersensitive antigens (Valentine-Thon et al., 2006; Pichler & Tilch, 2004a). Also, appropriate decisions or alternative methods of treatment can be developed for patients that may have hypersensitivities to antigens found in bacteria or prostheses (Valentine-Thon et al., 2007; Stejskal et al., 2006; Hallab et al., 2005). MELISA® testing may prove to be an invaluable tool for the determination of contributing factors to persistent conditions so appropriate treatment may be achieved.

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<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Method of Detection</th>
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<tbody>
<tr>
<td>I</td>
<td>An immediate reaction that can result in an anaphylactic reaction</td>
<td>Provocation test, Skin test, IgE RAST</td>
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<tr>
<td>II</td>
<td>Cytotoxic reaction mediated by IgM and IgG antibody responses to host tissue</td>
<td>IgG serum test</td>
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<tr>
<td>III</td>
<td>IgG and IgM antibodies form immune complexes with antigens in the blood</td>
<td>IgG serum test</td>
</tr>
<tr>
<td>IV</td>
<td>Delayed reaction that is mediated by memory T cells</td>
<td>Skin test, MELISA®</td>
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Comparing Hypersensitivity to Toxicity

Hypersensitivity and toxicity appear to be two related events, yet they are two distinct responses that can occur in isolation of each other. Hypersensitivity reactions can transpire after acute contact with an antigen, whereas toxicity is a harmful reaction that may occur with exposure to excessive amounts of a substance (Hallab et al., 2005). To explain further, hypersensitivity can occur without a toxic reaction because a hypersensitive response is not dose-dependent and can take place with very low levels of an antigen (Stejskal & Stejskal, 1999). Therefore, if a patient presents with a hypersensitivity reaction, even small amounts of the antigen may result in health problems emphasizing the importance of antigen avoidance.

Conclusion

Hypersensitivity reactions are common yet they aren’t often considered in treatment regimens. Untreated hypersensitivities can contribute to a myriad of conditions including autoimmune diseases (Valenta et al., 2009), irritable bowel syndrome (Spiller, 2004), asthma (Fernandez-Nieto et al., 2006), and psychiatric illnesses (Roy-Byrne et al., 2008). By utilizing appropriate techniques to determine the existence of hypersensitivity reactions, patients can become better aware of which antigens to avoid. Ultimately, by minimizing exposure to potential antigens patients may decrease the likelihood of developing chronic diseases and therefore increase their quality of life.

References


