Hypersensitivity: T Lymphocyte-mediated (Type IV)

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Five types of hypersensitivity reactions have been described: types I, II, III and V depend on the interaction of antigen with antibody and have been termed immediate; type IV depends on the interaction of antigen with T lymphocytes and has been called delayed-type hypersensitivity, or DTH. The DTH reaction involves cellular activation of T-helper cells (CD4+) and/or cytotoxic T cells (CD8+ CTLs). Subsequent cytokine secretion gives rise to distinct pathologies. In many cases, sustained release of antigen and continued activation of sensitized T cells results in amplification of responses that provoke excessive macrophage activation. These events are the basis for development of a broad range of inflammatory pathologies which range from erythema and oedema to granuloma formation, to extended development of fibrosis and even tissue necrosis.

Introduction

The type IV hypersensitivity, or delayed-type hypersensitivity (DTH), is characterized by its observed time course in which the reactions take approximately 12–24 h to develop. Because the response is not immediate, it is referred to as ‘delayed-type’ hypersensitivity. The reactivity persists for 2–3 days, and is dependent on the direct action of T lymphocytes which recognize processed and presented antigen. Unlike the other hypersensitivities, the type IV hypersensitivity is not antibody-mediated but rather defined as a T lymphocyte-mediated response that can occur in response to a variety of different stimuli. It is most commonly seen as a response to exposure to antigens from certain types of microorganisms and as a reaction against specific chemical compounds (allergic contact hypersensitivity). The T lymphocyte-mediated reaction requires the mobilization and activation of effector cells (Kobayashi et al., 2001). Diseases such as tuberculosis and leprosy are clinical examples of DTH where tissue injury is primarily due to the vigorous immune response to released antigens rather than damage due to the inciting pathogen itself. DTH reactions play a role in granulomatous diseases of unknown aetiology, such as sarcoidosis. As an understanding of the role of T cells in the development of hypersensitivities expands, it is becoming clear that deleterious pathologies occurring during many autoimmune disorders, as well as those seen during acute allograft rejection, also have a strong aetiological DTH-based component. See also: Hypersensitivity: Immunological Microorganisms

Repeat exposure to a variety of microorganisms, either living or dead, or to antigens derived from these microorganisms, has long been recognized as inducing a DTH response. Typically, infection with these microorganisms is associated with tissue granuloma formation at the site of infection. Many of the organisms associated with DTH are intracellular pathogens. One characteristic of these organisms is their resistance to killing by activated
macrophages. See also: Immune Mechanisms against Intracellular Pathogens

The microorganism classically associated with the DTH response is *Mycobacterium tuberculosis*, the causative agent of tuberculosis (North and Jung, 2004). The reaction was first demonstrated at the turn of the century by injecting a filtrate of killed *M. tuberculosis* into scariﬁed areas of skin. Mantoux modiﬁed the procedure by using intradermal inoculation, allowing greater sensitivity and quantiﬁcation of the reaction. An individual sensitized to tuberculosis through exposure or infection develops CD4+ lymphocytes speciﬁc for Mycobacterial antigens. Currently, the most common antigen used for the tuberculin DTH testing in tuberculosis is puriﬁed protein derivative (PPD). Intradermal injection of the PPD tuberculin results in activation of sensitized CD4+ T cells. This is followed by secretion of cytokines which cause recruitment and activation of macrophages (Chu et al., 1992). The ﬁnal outcome is a localized reactivity manifested by erythema and induration within 48 h (Figure 1). Antigens from other mycobacteria are also associated with DTH, including *Mycobacterium leprae*, *Mycobacterium kansasii* and *Mycobacterium avium-intracellulare*. In addition, injection of an attenuated strain of *Mycobacterium bovis*, Bacille Calmette–Guérin (BCG), can induce a subsequent DTH response to PPD. See also: Tuberculosis

Systemic mycoses can give rise to clinical manifestations of DTH. Infection with a variety of fungi is also associated with DTH (Almeida, 2008). These include *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Coccidioides immitis*, as well as *Trycophyton* and *Candida* species. Likewise, certain viral infections have also been associated with the reaction, including smallpox, measles and *Herpes simplex*. In fact, Jenner ﬁrst described the phenomenon of DTH in 1798. He called it the ‘reaction of immunity’. Lymphangigranularem, an infection due to the LGV serovars of *Chlamydia trachomatis*, also is associated with a DTH response to antigen injection, called the Frei test. Finally, several protozoal diseases, notably those due to *Leishmania* and *Schistosoma* species, are associated with DTH reactions. See also: Chlamydiae; Fungal Pathogens of Humans; Herpesviruses (Human); Protozoan Pathogens of Humans

Histologically, the initial reaction in DTH from microbial or pathogenic antigens occurs by 4 h and consists of an accumulation of neutrophils around the postcapillary venules at the site. This reaction subsides and is replaced, after approximately 12 h, by mononuclear cells, consisting of T lymphocytes and blood monocytes, which are also distributed around the venules in a perivascular cuff (Dvorak et al., 1974). In addition, the endothelial cells lining the venules become enlarged and porous to blood proteins. Fibrinogen leaking into surrounding tissue is converted to ﬁbrin. The accumulation of mononuclear cells plus ﬁbrin causes induration at the site of exposure, the hallmark of DTH. See also: Mononuclear Phagocytic System

Clinically, this reaction is apparent as erythema and oedema within the ﬁrst 12 h after antigen exposure. As the reaction continues, induration can be felt, with the maximum reaction occurring from 24 to 72 h after exposure to the antigen. The extent of palpable induration, rather than erythema, is the best quantitative measurement of DTH. The slower development of induration distinguishes DTH clinically from the Arthus reaction. In the latter case, repeated injections of antigen into the skin result in marked inﬂammation at the site of injection within hours and are due to the deposition of immune complexes at the site of injection with subsequent complement activation. Hence, it is fundamentally different from DTH (Poulter et al., 1982).

Allergic Contact Dermatitis (Cutaneous, Nonpathogenic-associated DTH)

Another common form of DTH is allergic contact dermatitis. Clinically, reactivity manifests as a pruritic, vesicular rash. The compounds associated with this disease are usually molecules of low molecular mass. Although some agents, such as 2,4-dinitro-1-chlorobenzene (DNCB), may cause hypersensitivity after one exposure and are sensitizing in virtually all immunocompetent individuals, most require repeated skin contact to induce a subsequent reaction. The agents of contact sensitivity are of too low a molecular mass to act as antigens on their own. Instead, they act as hapten by binding to tissue proteins in the skin. Normally, the immune system is tolerant to these skin proteins. When bound, our own proteins serve as carriers, thus rendering the foreign hapten immunogenic. The antigen is the hapten–tissue protein complex. See also: Antigen-presenting Cells; Hapten

Contact hypersensitivity is initiated by normal surveillance and antigen-presenting cell (APC) functioning of Langerhans cells and dendritic cells present in the

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Figure 1  Erythema and induration at the site of intradermal injection of PPD in a patient previously infected with *M. tuberculosis*. A positive reaction of more than 10 mm indicates reactivity in immune competent individuals. From Roitt (1998).
epidermis (Toebak et al., 2009). These cells subsequently interact with the CD4+ lymphocytes to initiate a cascade of events culminating in development of pathology. Reactivity over time can be deleterious, with accumulation of activated lymphocytes populating the dermis, and subsequent secretion of cytokines due to recognition of antigen in extravascular spaces (Figure 2).

Of interest, hypersensitivity reactions that cause contact dermatitis can be enhanced after exposure to soluble antigens by mixing them with a variety of agents that increase their immunogenicity. These are called adjuvants. Freund’s complete adjuvant, consisting of killed M. tuberculosis emulsified in mineral oil, is one of the most commonly employed adjuvant used in research. See also: New Generation Vaccine Adjuvants

The most common causes of contact dermatitis are the Toxicodendron plants, including poison ivy, poison oak and poison sumac. Other frequent offenders are paraphenylenediamine and ethylenediamine, formaldehyde, potassium dichromate, nickel and rubber. The topical antibiotic neomycin sulfate, as well as sulfonamides, may also induce this reaction (Posadas and Pichler, 2007). Cosmetics and soaps may also contain agents that cause contact hypersensitivity. Contact with the agent in a sensitized individual promotes activation of cytotoxic lymphocytes, resulting in itching, erythema, vesicles or eczema within 48 h. Chronic contact dermatitis can progress to dyspigmentation or lichenification. If not treated, necrosis of skin tissue is likely to occur. See also: Allergy; Skin: Immunological Defence Mechanisms

Granulomatous Hypersensitivity

In many cases, the antigen persists for extended periods of time, resulting in a pathology characterized as granulomatous hypersensitivity. This is best exemplified by the histologic manifestation of M. tuberculosis infection (Flynn and Chan, 2001; Hunter et al., 2007). Strong CD4+ DTH response and CD8+ cell-mediated immunity are essential for organism containment and control of dissemination (Figure 3). In essence, a depot of antigen exists that maintains cellular activation. Macrophages are continuously recruited in developing granulomas. Caseation results from destruction of insufficiently activated macrophages harbouring bacilli. The lesions grow when intracellular multiplication produce sufficient antigens to further activate lymphocytes; caseous centers progressively enlarge to destroy adjacent tissues (North and Jung, 2004; Actor et al., 2007). Other mycobacterial agents also elicit hypersensitive responses; T-cell responses to M. leprae are responsible for borderline leprosy with swollen skin lesions and hypopigmentation found at nerve endings (Spierings et al., 2000).

Granulomatous reactions are prominent in many parasitic infections, particularly those where deposition of eggs introduces a source of antigen that is not readily cleared from immune recognition. Liver damage is readily seen in Schistosoma species infections. Likewise, diseases caused by fungal agents (e.g. Histoplasmosis, Coccidioidomycosis, Cryptococcus, Blastomycosis, Pneumocystis), by various filamentous bacteria (e.g. Actinomyces and Nocardiosis) also have a dependent DTH-based reaction...
that leads to pathogenesis. Other granulomatous inflammatory disorders of unknown aetiologies, for example, sarcoidosis and Crohn disease, have been shown to have a strongly associated DTH element contributing to manifestation of pathology. See also: Crohn Disease and Ulcerative Colitis; Immunity to Fungi; Immunity to Parasitic Worms

Autoimmune Disease

T lymphocyte-directed hypersensitive responses can result in clinical manifestation of autoimmune disease when this response is directed against self-antigens. This failure of self-tolerance in essence is the basis for development of many aspects of autoimmunity. Indeed, it is becoming more appreciated that activation of autoreactive T cells that recognize self-antigens on organs can lead to direct tissue damage. Although the mechanisms underlying all autoimmune diseases are not fully elucidated, genetic polymorphisms of major histocompatibility complex (MHC) class II genes (alleles of major histocompatability HLA (histocomplatability leukocyte antigen)-DR or HLA-DQ) are associated with increased autoimmune manifestation. For example, during multiple sclerosis, T cells reactive to myelin basic protein destroy the protective layer surrounding axons, thus eliminating effective transfer of signals through nerves. In type I diabetes and insulin-dependent diabetes mellitus (IDDM), T cells contribute to destruction of islet cells leading to failure of insulin production. In these examples, self-antigens presented on the surface of APCs are recognized by the T-cell receptor, resulting in subsequent activation of the T lymphocyte. The signalling cascade and release of cytokines and chemokines (detailed below) which drive the underlying pathology of autoimmune disease.

In many cases these autoreactive T cells coexist with autoantibody responses, further exacerbating disease development. Autoreactive T cells to thyroid microsomal antigens and autoantibodies to thyroglobulin are prominent in Hashimoto thyroiditis, with resultant destruction of the thyroid gland. Infiltrating autoreactive T cells in synovium following deposition of immune complexes to rheumatoid factors in rheumatoid arthritis lead to joint inflammation and subsequent destruction of cartilage and bone. See also: Autoimmune Disease: Aetiology and Pathogenesis; Autoimmune Disease: Mechanisms; Autoimmune Disease: Pathogenesis

Allograft Rejection

Grafts made between genetically different individuals (allografts) are only tolerated when major histocompatibility antigens are matched. Alloreactivity is defined by stimulation of host T-cell responses against foreign HLA antigens, with helper and cytotoxic T lymphocytes and macrophage-mediated DTH responses mediating allograft rejection (Ode-Hakim et al., 1996; Youssef et al., 2002).

Acute graft rejection can occur 10–30 days after tissue implantation when T cells responding to donor tissue antigens are amplified and activated. Chronic rejection
Mechanisms

24 h. IFN peaks after 24 h, whereas TNF is first seen significantly after can be detected in as few as 12 h after antigen exposure and necrosis factor (TNF) and IL-17 (Iwakura include interleukin-2 (IL-2), interferon g, and peaks shortly afterwards. Local blood vessels are dir-

Cellular and Immunological

Mechanisms

DTH cannot be transferred to other individuals through serum. In this way, it differs fundamentally from the other forms of immunological hypersensitivity. However, DTH can be transferred by cells, specifically T lymphocytes, from one host to another, as long as these hosts are genetically identical.

Immunologically, DTH occurs when a previously sen-
sitized individual is re-exposed to the antigen. Antigen is presented by APCs, including resident Langerhans cells, vascular endothelial cells and monocytes, to circulating T lymphocytes. Although CD8+ T lymphocytes may play a role in some instances of DTH, CD4+ T lymphocytes (helper phenotype) are more commonly found. Current research indicates that T-helper (T_{H1}) cells initially recruited differentiate into type 1 (T_{H1}) and type 17 (T_{H17}) pheno-
types. On secondary exposure, elicitation occurs, involving activation of both T_{H1} and T_{H17} cells to release cytokines and activate macrophages. Both T lymphocytes and the activation of macrophages are important to the patho-
genesis of DTH and both act through the secretion of various cytokines. In addition, CD8+ lymphocytes are also expanded during the sensitization stage. Damage of tissue will occur when these cells are further activated, leading to cell-mediated cytotoxicity-associated responses. See also: Graft Rejection: Mechanisms

In vitro Correlates

There are several in vitro tests that appear to correlate well with the DTH response. One is the assay for macrophage migration inhibition factor (MIF). Originally found to reduce the amount of random migration of macrophages, MIF is now known to amplify inflammatory responses to antigen and is expressed by T lymphocytes and macrophages. In the original assay, unstimulated peripheral blood mononuclear cells (PBMC) were placed in capillary tubes within chambers containing supernatant media from cells previously stimulated with antigen. The degree to which the cells failed to migrate out of the capillary tubes was a measure of MIF. Recently, a chemotaxis device consisting of two chambers divided by a filter with 5 μm pores has been used. Mononuclear cells, including monocytes, are placed in the top chamber, whereas supernatant from antigen-stimu-

factor (GM-CSF) stimulate further production of macro-

phages (Higashi et al., 1995). These factors upregulate expression of adhesion molecules on endothelium, as well as increase blood vessel permeability, which allows both accessory cells and fluids to enter the site (causing oedema). The evidence that these cytokines are associated with the clinical inflammatory response is supported by the facts that the reaction can be inhibited by the injection of cytokines that inhibit their production, such as IL-10; that the injection at the site of supernatants from in vitro-stimulated peripheral blood lymphocytes boosts the clinical response; and that many elements of the DTH response can be induced by the intradermal injection of the cyto-

tinues. See also: Cytokines; Tumour Necrosis Factors

A variety of other inflammatory molecules are produced during a DTH response, including monocyte chemotactic protein-1 (MCP-1), RANTES (regulated on activation, normal T expressed and secreted protein), the adhesion molecules E-selectin and intercellular adhesion molecule-1 (ICAM-1) and macrophage inflammatory protein 1α (MIP-1α; CCL3) and MIP-1β (CCL4), chemokines that appears to attract both granulocytes and lymphocytes to the site of antigen exposure and subsequently contribute greatly to neutrophilic inflammation. See also: Inflam-
mation: Acute; Inflammation: Chronic

During a DTH reaction, cytokines are secreted at the site of antigen exposure in a well-regulated sequence (Buchanan and Murphy, 1997). Cytokines found to be present in lesions include interleukin-2 (IL-2), interferon γ (IFNγ), tumour necrosis factor (TNF) and IL-17 (Iwakura et al., 2008). IL-2 can be detected in as few as 12 h after antigen exposure and peaks after 24 h, whereas TNF is first seen significantly after 24 h. IFNγ of particular importance in driving macrophages to release inflammatory mediators, is first detected after 24 h and peaks shortly afterwards. Local blood vessels are direct-

ly affected through the release of TNFα and TNFβ, whereas additional lymphocyte-released factors, such as IL-3 and granulocyte–macrophage colony stimulating

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indicates activation and cellular expansion. See also: Macrophages

Finally, since DTH reactions are associated with the production of a variety of cytokines, these can be measured in the supernatants of PBMC stimulated with antigen. Cytokines associated with DTH that are now commonly measured include IL-2, IFNγ and TNFα, although assays for many others are available. Originally, assays which measured the biological activity of these cytokines were used. Currently, enzyme-linked immunosorbent assays (ELISAs), which directly measure the concentration of cytokine present in the supernatant by the extent of binding to specific monoclonal antibodies, are commonly employed. Gene expression of cytokines using reverse transcriptase polymerase chain reaction methods (RT-PCR) is now a powerful tool to determine specific activation, with multiple markers readily identified for phenotypic cell response. See also: Cytokine Assays; Enzyme-linked Immunosorbent Assay; Reverse Transcription and PCR

Medical Uses and Consequences of Delayed-type Hypersensitivity

Detection of infection

One of the most common uses of the DTH reaction is to determine whether an individual is infected with a particular microorganism. It is important to realize that although the finding of DTH to a particular microorganism may indicate that the individual is currently infected with that agent, it may also indicate past exposure to a cleared infection. It does not indicate when the individual was infected, either recently or remotely, nor does it imply that the individual is ill from the infection. In some infections, such as the endemic mycoses, a DTH response suggests that the patient is controlling their infection. Moreover, since the ability to mount a DTH response to an antigen is a reflection of an intact cellular immune response, many patients with suppressed cellular immunity may be infected and ill from the microorganism in question and yet not manifest a DTH response.

DTH testing is most commonly employed for the diagnosis of tuberculosis in individuals who have not been previously immunized with BCG. Typically, 0.1 ml of 5 TU (tuberculin units) of PPD is injected intradermally (Man
toux method) into the volar aspect of the forearm (Sokal, 1975). The test is usually read after 48 h, but the reaction may not reach a peak until 72 h after injection. The extent of induration should be read in two diameters at right angles and the two values averaged. Because the margins of induration are often indistinct, there can be great variability in determining the size of the reaction, even with the same reader. To overcome this, it has been recommended that a ballpoint pen be used to draw a line towards the area of induration, using moderate pressure. When resistance to movement is felt, this is marked as the outer margin of induration. The technique is repeated in three other quadrants and the two diameters determined. See also: Tuberculosis

A positive response to PPD injection varies depending on the individual being tested. A reaction of 5 mm is taken as positive in immunosuppressed individuals, such as those infected with human immunodeficiency virus (HIV) (Dolan et al., 1995). For those without immunosuppressive conditions but who are at high risk for contracting tuberculosis, such as individuals from countries with a high prevalence of disease, 10 mm is considered positive and indicative of infection. Finally, for those individuals without any risk for tuberculosis, the reaction must be > 15 mm to be looked upon as positive. See also: Human Immuno
deficiency Viruses (HIV)

Intradermal (skin) tests are available for diagnosis of other bacterial agents, such as those responsible for leprosy, lymphgranuloma venereum, bartonellosis, scarlet fever and anthrax. Regarding leprosy, a positive skin test is diagnostic for tuberculoid disease, with active cell-mediated immunity. A negative test in an infected individual suggests lepromatous leprosy, with a T-cell response that is skewed away from IFNγ production. In addition to bacterial agents, the test can be similarly engineered to determine whether an individual is infected with endemic fungi. For example, testing for reactivity against coccidioidin or spherulin will assist in diagnosis of coccidiomycosis. Similar tests are available for Candidiasis, Histoplasmosis and Dermatophyto
dy due to Trichophyton spp. Likewise, tests are available for parasitic infections, such as for organisms responsible for Leishmaniasis and Oncoeceriasis. In these cases, the test is considered as positive if ≥ 5 mm of palpable induration occurs 48 h after antigen injection. The most common use of these tests is epidemiological. The rate of positivity is used to determine the prevalence of infection in a certain geographical area.

Anergy

Another important use of DTH testing is to determine whether the cellular immune response is intact. Failure to develop DTH responses to antigens from microorganisms with which the individual is already infected is termed anergy (Schwartz, 2003). Anergy may be specific to one agent, or more generalized. To test for generalized anergy, antigens to which there has been universal exposure are employed as testing agents. These include antigens from Candida, Trichophyton and mumps. See also: Mumps Virus

A common example of generalized anergy occurs in patients infected with HIV. Lack of DTH reactions to mumps, Candida and Trichophyton antigens predicts a more rapid progression to immunodeficiency and death in such patients independently of other factors, including the number of peripheral blood CD4+ lymphocytes or the clinical stage of the patient. Moreover, anergy to these
Detection of Allergic Contact Dermatitis

The clinical manifestations of allergic contact dermatitis are not easily distinguishable from other causes of dermatitis in which an eczematous reaction occurs. One useful technique for assessing whether an eczematous reaction is due to contact dermatitis is through patch testing (Ahmed and Blose, 1983; Lockey, 1995). First devised in 1896, the technique consists of placing the suspected offending chemicals in a series of patches on normal-appearing skin, usually on the back. After covering with a nonabsorbent adhesive patch, the area is examined 48 h later. A positive reaction consists of a localized area of eczema, appearing as erythema and papules at the site of the patch.

Treatment

Skin testing for DTH and patch testing for allergic contact dermatitis do not usually lead to any untoward medical consequences other than mild local irritation. Occasionally, local reactions can be more significant. Then, topical administration of corticosteroids is helpful. Rarely, for severe local reactions, systemic corticosteroids may be needed. Very rarely, immediate allergic reactions to skin test reagents have been reported. For this reason, patients should remain in the clinic for at least 20 min after a test is applied. For mild immediate reactions, such as hives, an oral antihistamine can be given. For more severe reactions, such as anaphylaxis, adrenaline and parenteral antihistamines should be administered and the patient should be monitored closely thereafter. See also: Hypersensitivity: Anaphylactic (Type I)

References


**Further Reading**


