LECTURE: 11

Title: IMMUNOLOGICAL MECHANISM IN TISSUE DAMAGE DELAYED HYPERSENSITIVITY- CELL MEDIATED CYTOTOXICITY TYPE-IV HYPERSENSITIVITY

LEARNING OBJECTIVES:

The student should be able to:

- Describe type IV hypersensitivity reaction including the most common cytokines.
- Identify the classical example of type IV reaction.
- List some common antigens such as:
  - Mycobacterium.
  - Insects bites and stings.
  - Metals (nickel, coins, and jewelry).
  - Cosmetics, and paints
  - Drug allergies.
  - Plants "Poison Oak and Ivy"

- List the two cell-mediated facets such as:
  - Delayed type hypersensitivity Tdth (CD4$^+$ cells) cells.
    - ♦ Secreting γ-interferon (activate macrophages, and recruit the immigration of more cells into the area).
    - ♦ Secret IL-2 is a mitogenic factor: it stimulates the growth of activated T cells.

  - Cytotoxic Tc (CD8$^+$ cells) cell responses.
    - ♦ Perforin: function as C9 complement protein.
- List some clinical features for type IV hypersensitivity such as:
  - Contact dermatitis.
  - Some autoimmune diseases.
  - Tuberculin skin test reaction.
  - Hypersensitivity pneumonitis (extrinsic allergic alveolitis).
  - Cutaneous basophil hypersensitivity (Jone-Mote- reaction).

- **List some in vivo and in vitro tests for type IV diagnosis.**

Hypersensitivity – Type IV

- There are three varieties of Type IV hypersensitivity: contact, tuberculin, and granulomatous.

- Langerhans' cell internalizes and process epicutaneously applied hapten and present it to antigen-specific T cells.

- Cytokines produced by immune-competent skin cells (e.g. keratinocytes, Langerjhans' cells, T cells) recruit antigen-non-specific T cells and macrophages.

- Tuberculin-type hypersensitivity is useful as a diagnostic test for exposure to a number of infectious agents.

- In granulomatous reactions there is a balance between protective immunity and T cell mediated tissue damage to insoluble antigen. A good example of this is seen in tuberculoid leprosy.

- Persistence of antigen leads to differentiation of macrophages to epithelioid cells, and fusion to form giant cells. The whole pathological response is termed a granulomatous reaction and it results in tissue damage.

- Granuloma formation is driven by T cell activation of macrophages, and is dependent on tumor necrosis factor (TNF).

According to the Coombs' and Gel classification, Type IV or delayed hypersensitivity reactions take more than 12 hours to develop and involve cell-mediated immune reactions rather than antibody responses to antigens. They serve more widely as a model of T cell-mediated inflammatory responses to either exogenous or autoantigens. When the exogenous antigen is applied to the epidermis or injected intrademally in a sensitized individual, antigen specific T cells stimulate a local inflammatory response over 24-72 hours. If the antigen is an organ-specific self antigen, autoreactive T cell may produce

localized cellular inflammation and autoimmune disease, such as Type I diabetes. Some hypersensitivity reactions may straddle this definition with a rapid antibody-mediated phase and a later cell-mediated phase. For example, the late phase IgE-mediated reaction may peak 12-24 hours after contact with an allergen, and cells, such as T helper (TH) 2 cells and eosinophils, are involved as well as IgE.

Unlike other forms of hypersensitivity, Type IV hypersensitivity cannot be transferred from one animal to another by serum, but can be transferred by T cells, particularly CD4 TH1 cells in mice. Therefore it can occur in antibody deficient humans, but is lost with the decline in CD4 T cells in HIV/AIDS Type IV hypersensitivity reflects the presence of antigen-specific CD4 T cells and is associated with protective immunity against intracellular and other pathogens. However, there is not a complete correlation between Type IV hypersensitivity and protective immunity. The T cells responsible for the delayed response have been specifically sensitized by a previous encounter with the antigen, and act by recruiting macrophages and other lymphocytes to the site of the reaction.

Three variants of Type IV hypersensitivity reaction are recognized (Figure-1). Contact hypersensitivity and tuberculin type hypersensitivity both occur within 72 hours of antigen challenge. Granulomatous hypersensitivity reactions develop over a period of 21-28 days; the granulomas are formed by the aggregation and proliferation of macrophages, and may persist for weeks. In terms of its clinical consequences, this is by far the most serious type of Type IV hypersensitivity respone. Note that more than one types of reaction may follow a single antigenic challege, and that the reactions may overlap.

The three types of delayed hypersensitivity were originally distinguished according to the reaction they produced when antigen was applied directly to the skin (epicutaneously) or injected intradermally. The degree of the response is usually assessed in animals b measuring thickening of the skin. The local response is also accompanied by a variety of systemic immune responses, such as T cell proliferation and synthesis of cytokines including interferon-γ (IFNγ).

CONTACT HYPERSENSITIVITY

Contact hypersensitivity is characterized by an eczematous reaction at the point of contact with an allergen (Figure-2). It is often seen following contact with agents such as nickel, chromate, rubber accelerators and pentadecacatechol (found in poison ivy). Contact with irritants that damage skin by toxic mechanisms not mediated by hypersensitivity can also produce eczema. Although the initial reactions are different, the inflammatory events following application of irritants and allergens show similarities.

The immunologically active portions of the agents listed above are called haptens. Haptens are too small to be antigenic by themselves, having a molecular weight often less than 1 kDa. They penetrate the epidermis and conjugate, most often covalently, to body proteins. The sensitizing potential of a hapten cannot reliably be predicted from its chemical structure, although there is some correlation with the number of haptens attached to the carrier and the ability of the molecule to penetrate the skin. Also, certain

contact allergens have unsaturated carbon bonds and are easily oxidized. Some haptens, such as dinitrochlorobenzene (DNCB), sensitize nearly all individuals and can be used to asses cell-mediated immunity. Epicutaneously applied DNCB binds to epidermal proteins and to MHC-linked peptides through the $-NH_2$ groups of lysine.

Langerhans' cells and kerationocytes have key roles in contact hypersensitivity

The Langerhans' cell is the principal antigen-presenting cell

Contact hypersensitivity is primarily an epidermal reaction, and the dendritic Langerhans' cell, located in the suprabasal epidermis, is the principal antigen-presenting cell (APC) involved (Figure-3). Langerhans' cells are derived from bone marrow and express CD1, MHC class II antigens and surface receptors for Fc and complement. Electron microscopy shows Birbeck granules, organelles derived from cell membrane and specific for the cell. Langerhans' cells are inactivated b ultraviolet B, which can thus prevent or alleviate the effectors of contact hypersensitivity.

In vitro, Langerhans' cells act as APCs and are more potent in this regard than monocytes. Langerahans' cells take up hapten-modified proteins by micropinocytosis and under the influence of interleukin (IL-1) and TNF form keratinocytes and other cells undergo maturation, increase the expression of MHC and co-stimulatory molecules and migrate to draining lymph nodes.

Keratinocytes produce a range of cytokines important to the contact hypersensitivity response

Keratinocytes provide the structural integrity of the epidermis and have a central role in epidermal immunology. They may express MHC class II molecules and intercellular adhesion molecule-1 (ICAM-1) in the cell membrane. They can also release cytokines including IL-1, IL-3, IL-6, IL-8, granulocyte-macrophage-colonystimulating factor (M-CSF), TNFα, transforming growth factor-α (TGFα). IL-3 can activate Langerhans' cells, co-stimulate proliferative responses, recruit mast cell and induce the secretion of immunosuppressive cytokines (e.g. IL-10 and TGFβ). These latter dampen the immune response and induce clonal anergy (immunological unresponsiveness) in TH1 cells.

Keratinocytes can be activated by a number of stimuli, including allergens and irritants. Activated keratinocytes produce immunostimulatory cytokines such as TNFα and GM-CSF which activate Langerhans' cells. Some antigens, such as urushiol in poison ivy, may directly induce TNFα and IL-8.

A contact hypesensitivity reaction has two stages: sensitization and elicitation

Sensitization produces a population of memory T cells
Sensitization takes 10-14 days in humans. Once absorbed, the hapten combines with a protein andis internalized by epidermal Langerhans' cells, which leave the epidermis and migrate as veiled cells through the afferent lymphatics to the paracortical areas of regional lymph nodes. Here they present processed hapten-protein conjugates (in

association with MHC class II molecules) to CD4$^+$ lymphocytes, producing a population of memory CD4$^+$ T cells (Figure-4). In addition to CD4 T cells, MHC class I-restricted CD8 T cells are important in contact hypersensitivity responses to some allergens in humans and mice. For example lipid-soluble urushiol from poison ivy can enter the cytoplasm of APCs and the MHC class I processing pathway, leading to the activation of allergen-specific CD8 T cell clones. These cause inflammation by direct cytolytic effect on epidermal cells or b the release of IFNγ.

Elicitation involves recuitmenet of CD4$^+$ lymphocytes and monocytes

The application of a contact allergen generally causes a modest decrease in Langerhans' cell numbers in the epidermis within hours of application. Antigen presentation by Langerhans' cells then occurs in skin and lymph nodes. TNFα and IL-1 form many cell types and from macrophages in particular, are potent inducers of endothelial cell adhesion molecules. These locally released cytokines produce a gradient signal for movement of mononuclear cells towards the dermo-epidermal junctions and epidermis. For the elicitation phase of contact hypersensitivity, see Figure-5.

The earliest histological change, seen after 4-8 hours, is the appearance of mononuclear cells around adnexae and blood vessels, with subsequent epidermal infiltration. Macrophages invade the dermis and epidermis by 48 hours. The number of cells infiltrating the epidermis and dermis peaks at 48-72 hours (Figure-6). Most infiltrating lymphocytes are CD4$^+$, with a few CD8$^+$. Less than 1% of infiltrating cells are antigen-specific memory CD4$^+$ TH1 cells. The later stages for T cell recruitment are Ag independent. Askenase has shown that IgM antibodies to the hapten produced by B-1 cells are important for Ag localization, to elicit the reaction. Experiments in gene-targeted mice show that selectins, ICAM-1 and the integrins, leucocyte functional antigen-1 (LFA-1) and very large antigen-4 (VLA-4), are all required for the elicitation of contact and delayed hypersensitivity.

The mechanisms of reactions to allergens and irritants share some features

TNFα, IFNγ and GM-CSF mRNA signals are indeed in Langerhans' cells within 30 minutes of topical application of either an antigen or irritant, and a tenfold increase in mRNA expression is found in 2-4 hours. Certain changes in mRNA transcription occur only after application of a hapten. These include an increase in the IL-1β mRNA signal by Langerhans' cells at 15 minutes, and upregulation of transcription by keratinocytes of IL-1α, macrophage inflammatory protein 2 (MIP-2), and interferon-induced protein 10 (IP-10) (Figure-7).

Chemical reagents applied to the epidermis can result in increased expression of ELAM-1 (endothelial leukocyte adhesion molecule) and VCAM-1 within 2 hours, and ICAM-1 within 8 hours, regardless of whether the individual is sensitive or not. ICM-1 is more prominent than VCAM-1 or ELAM-1: it is the ligand for LFA-1, found on lymphoid and myeloid cells, and is important for localizing these cells to the skin. Chemotactic cytokines and the 'beacon effect' of in-transit Langerhans' cell attract TH1 cells. Memory

T cells reside in dermal capillaries where they can trigger the reaction and recruit in a non-antigen-specific manner.

Suppression of the inflammatory reaction is mediated by a range of cytokines

The reaction wanes after 48-72 hours; macrophages and keratinocytes produce PGE, which inhibits IL-1 and IL-2 prodiction; T cells bind to activated keratinocytes and the hapten conjugate undergoes enzymatic and cellular degradation. Downregulation is assisted by the following mechanisms:

- Migration-inhibitory lymphokines prevent spread of the inflammatory reaction.

- TGFβ, from dermal mast cells, activated keratinocytes and lymphocytes, inhibits inflammation and blocks the proliferation effects of IL-1 and IL-2.

- IL-1, synthesized by keratinocytes following contact with allergens, inhibits oxidative metabolism in macrophages and depresses their production of pro-inflammatory mediators.

- IL-10 downregulates class II molecule expression, and suppresses cytokine production and antigen-specific proliferation by TH1 cells.

- External factors may also be involved: in mice UV light has shown to induce a specific inhibitor of IL-1 activity.

- Keratinocytes expressing class II molecules without co-stimulatory molecules cannot act as prime lymphocytes but, when haptenated and incubated with TH1 cells, can induce clonal anergy.

TUBERCULIN-TYPE HYPERSENSITIVITY

This form of hypersensitivity was originally described by Koch. He observed that if patients with tuberculosis were injected subcutaneously with a tuberculin culture filtrate (antigens derived from the tubercle bacillus) they reacted with fever and generalized sickness. An area of hardening and swelling developed at the site of injection. Soluble antigens from a number of organisms, including *Mycobacterium tuberculosis, M. leprae* and *Leishmania tropica*, induce similar reactions in sensitive people. The skin reaction is frequently used to test for sensitivity to the organisms following previous exposure (Figure-8).This form of hypersensitivity may also be induced by non-microbial antigens, such as beryllium and zirconium.

The tuberculin skin test reaction principally involves monocytes

The tuberculin skin test is an example of the recall response to soluble antigen previously encountered during infection. Following intradermal tuberculin challenge in a sensitized individual, antigen-specific T cells are activated to secrete IFNγ which activates macrophages to produce TNFα and IL-1. These proinflammatory cytokines and chemokines from T cells and macrophages act on endothelial cells in dermal blood

vessels to induce the sequential expression of the adhesion molecules E-selectin, ICAM-1 and VCAM-1. These molecules bind receptors on leucocytes and recruit them to the site of the reaction. The initial influx at four hours is of neutrophils, but this is replaced at 12 hours by monocytes and T cells. This infiltrate, which extends outwards and disrupts the collagen bundles of the dermis, increases to a peak at 48 hours. $CD4^+$ T cells outnumber $CD8^+$ cells by about 2:1. $CD1^+$ cells (Langerhans-like cells, but lacking Birbeck granules) are also found in the dermal infiltrate at 24 and 48 hours, and a few $CD4^+$ cells infiltrate the epidermis between 24 and 48 hours.

Monocytes constitute 80-90% of the total cellular infiltrate. Both infiltrating lymphocytes andmacrophages express MHC class II molecules, and this increases the efficiency of activated macrophages as APCs. Overlying keratinocytes express HLA-DR molecules 48-96 hours after the appearance of the lymphocytic infiltrate. These events are summarized in Figure-9.

Macrophages are probably the main APCs in the tuberculin hypersensitivity reaction. However, there are $CD1^+$ cells in the dermal infiltrate, which suggests that Langerhans' cells or indeterminate dendritic cells may also participate. The circulation of immune cells to and from the regional lymph nodes is thought to be similar to that for contact hypersensitivity. The tuberculin lesion normally resolves within 5-7 days, but if there is persistence of antigen in the tissues it may develop into a granulomatous reaction.

Tuberculin-like delayed type hypersensitivity (DTH) reactions are used practically in two ways. First, reaction to soluble antigens from a pathogen demonstrates past infection with that pathogen. Thus, tuberculin reactivity confirms past infection with M. tuberculosis, but not necessarily active disease. DTH responses to frequently encountered microbes are a general measurement of cell-mediated immunity. This can be tested with intradermal injection of single antigens from common pathogens, or a multi-puncture device which delivers seven common microbial antigens in a standardized fashion. Loss of recall responses to specific antigens occurs in a wide range of diseases and infections which impair T cell function, and during therapy with corticosteroids or immunosuppressive agents.

GRANULOMATOUS HYPERSENSITIVITY

Granulomatous hypersensitivity is clinically the most important form of Type IV hypersensitivity, and causes many of the pathological effects in diseases that involve T cell-mediated immunity. It usually results from the persistence within macrophages of intracellular microorganisms or other particles that the cell is unable to destroy. On occasion it may also be caused by persistent immune complexes, for example in allergic alveolitis. This process results in epithelioid cell granuloma formation.

The histological appearance of the granuloma reaction is quite different from that of the tuberculin-type reaction. However, they often result form sensitization to similar microbial antigens, for example the antigens of M. tuberculosis and M. leprae (Figure-10). Immunological granuloma formation also occurs in the sensitivity reactions to zirconium and beryllium, and in sarcoidosis, although in the latter the antigen is

unknown. Foreign-body granuloma formation occurs with talc, silica and a variety of other particular agents. In this case macrophages are unable to digest the inorganic matte. These non-immunological granulomas may be distinguished by the absence of lymphocytes in the lesion.

Epithelioid cells and giant cells are typical of granulomatous hypersensitivity

Epithelioid cells – These cells are large and flattened with increased endoplasmic reticulum (Figure-11). They are derived from activated macrophages under the chronic stimulation of cytokines; they continue to secrete TNF and thus potentiate continuing inflammation.

Giant cells – Epithelioid cells may fuse to form multi-nucleate cells (Figure-12), sometimes referred to as Langhans' giant cells (not to be confused with the Langerhans' cell discussed earlier). Giant cells have several nuclei, but these are not at the centre of the cell. There is little endoplasmic reticulum, and the mitochondria and lysosomes appear to be undergoing degeneration. The giant cell may therefore be a terminal differentiation stage of the monocyte/macrophage line.

The granuloma contains epithelioid cells, macrophages and lymphocytes
An immunological granuloma typically has a core of epithelioid cells and macrophages, sometimes with giant cells. In some diseases, such as tuberculosis, this central area my have a zone of necrosis, with complete destruction of all cellular architecture. The macrophage/epithelioid core is surrounded by a cuff of lymphocytes, and there may also be considerable fibrosis (deposition of collagen fibres) caused by proliferation of fibroblasts and increased collagen sysnthesis. Examples of granulomatous reactions are the Mitsuda reaction to M. leprae antigens (Figure-12) or the Kveim test, where patients suffering from sarcoidosis react to (unknown) splenic antigens derived from other sarcoid patients. The three types of delayed hypersensitivity are summarized in Figure-13.

CELLULAR REACTIONS IN TYPE IV HYPERSENSITIVITY

Experiments with gene knockout (gko) mice have confirmed that T cells bearing αβ TCR rather than γδ TCT are essential for initiating delayed hypesensitivity reactions in response to infection with intracellular bacteria. Sensitized αβ T cells, stimulated with the appropriate antigen and APCs, undergo lymphoblastoid transformation prior to cell division (Figure-14). This forms the basis of the lymphocyte stimulation test. Lymphocyte stimulation is accompanied by DNA synthesis and this can be measured by assaying the uptake of radiolabeled thymidine, a nucleoside that is required for DNA synthesis. Lymphocytes from a patient are cultured with the suspect antigen to determine whether it induces transformation. It is important to stress that this is a test for T cell memory only, and does not necessarily imply the presence of protective immunity.

Following activation by APCs, T cells release a number of pro-inflammatory cytokines which attract and activate macrophages. These include IFNγ, lymphotoxin, IL-3 and GM-CSF. This ThH1-like pattern of cytokines is enhanced by activation of the T cells in the presence of IL-123, which is released by macrophages on exposure to bacterial products. IL-12 suppresses the cytokine response of TH2 cells. The role of individual cytokine can be analyzed in gko mice deficient for a single cytokine. For example, IFNγ gko mice are unable to activate macrophages and control infection with M. tuberculosis (Figure-15). In granulomatous reactions the activated macrophages become a major source of TNF and the granulomas develop by auto-amplification, with differentiation of macrophages into epithelioid cells (Figure-16, and 17). These secrete more TNF, stimulating further epithelioid cell formation, with the fusion of epithelioid cells resulting in the formation of giant cells (Figure-18). Granulomas fail to develop in the absence of TNF.

DISEASES MANIFESTING TYPE IV GRANULOMATOUS HYPERSENSITIVITY

There are many chronic diseases in man that manifest Type IV hypersensitivity. Most are due to infectious agents such as mycobacteria, protozoa and fungi, although in other granulomatous disease such as sarcoidois and Crohn's disease, no infectious agent has been established.

Important diseases in this respect include the following:

- Leprosy
- Tuberculosis
- Schistosomiasis
- Sarcoidosis
- Crohn's desease.

A common feature of these infections is that the pathogen presents a persistent, chronic antigenic stimulus. Activation of macrophages by lymphocytes may limit the infection, but continuing stimulation may lead to tissue damage through the release of macrophage products including reactive oxygen intermediates and hydrolases. Although delayed hypersensitivity is a measure of T cell activation, the infection is not always controlled, with the result that protective immunity and delayed hypersensitivity do not necessarily coincide. Therefore some subjects showing delayed hypersensitivity may not be protected against disease in the future.

Leprosy – Leprosy is divided clinically into three main types: tuberculoid, borderline and lepromatous. In tuberculoid leprosy, the skin may have a few –defined hypo-pigmented patches that show an intense lymphocytic and epithelioid infiltrate and no microorganisms. By contrast, the polar reaction of lepromatous leprosy shows multiple confluent skin lesions characterized by numerous bacilli, 'foamy' macrophages and a paucity of lymphocytes. Borderline leprosy has characteristics of both (Figure-19). In leprosy, protective immunity is usually associated with cell-mediated immunity, but this declines across the leprosy spectrum towards the lepromatous pole with a rise in non-protective anti- *M. leprae* antibodies.

The borderline leprosy reaction is a dramatic example of delayed hypersensitivity. Borderline reactions occur either naturally or following drug treatment. In these reactions, hypo-pigmented skin lesions containing *M. leprae* become swollen and inflamed (Figure-20), because the patient is now able to mount a delayed type hypesensitivity reaction. The histological appearance shows a more tuberculoid pattern with an infiltrate of IFNγ-secreting lymphocytes. The process may occur in peripheral nerves, where Schwann cells contain *M. leprae*; this is the most important cause of nerve destruction in this deasese. The lesion in borderline leprosy is typical of granulomatous hypersensitivity (Figure-20). In patients with a tuberculoid type reaction, T cell sensitization may be assessed *in vitro* by the lymphocyte stimulation test, using either whole or sonicated *M. leprae* as antigaen (Figure-21).

Tuberculosis – In tuberculosis there is a balance between the effects of activated macrophages controlling the infection on the one hand, and causing tissue damage in infected organs on the other. In the lung, granulomatous reactions lead to cavitation and spread of bacteria. The reactions are frequently accompanied by extensive fibrosis and the lesions may be seen in the chest radiographs of affected patients (Figure-22).

The histological appearance of the lesion is typical of a granulomatous reaction, with central caseous (cheesy) necrosis (Figure-23). This is surrounded by an area of epithelioid cells, with a few giant cells. Mononulear cell infiltration occurs around the edge.

Schistosomiasis – In schistosomiasis, caused by parasitic trematode worms (schistosomes), the host becomes sensitized to the ova of the worms, leading to a typical granulomatous reaction in the parasitized tissue mediated essentially by TH2 cells (Figure-24).

Sarcoidosis – Sarcoidosis is a chronic disease of unknown aetiology in which activated macrophages and granuloma accumulate in many tissues, frequently accompanied by fibrosis (Figure-25). The disease particularly affects lymphoid tissue, and enlarged lymph nodes may be detected in chest radiographs of affected patients (Figure-26). No infectious agent has been isolated, although mycobacteria have been implicated because of the similarities in the pathology.

One of the paradoxes of clinical immunology is that this disease is usually associated with depression of delayed hypersensitivity both *in vivo* and *in vitro*. Patients with sarcoidosis are anergic on testing with tuberculin: however, when cortisone is injected with tuberculin antigen the skin test are positive, suggesting that cortisone-sensitive T-suppressor cells are responsible for the anergy. Cortisone would normally suppress delayed hypersensitivity.

In sarcoidosis, granulomas develop in a variety of organs, most commonly the lungs, lymph nodes, bone, nervous tissue and skin. Patients may present acutely with fever and malaise, although in the longer term those with pulmonary involvement develop

shortness of breath caused by lung fibrosis. The diagnosis is often suggested by the clinical pattern and radiographic changes and confirmed by tissue biopsy. Angiotensin converting enzyme (ACE) and serum calcium are sometimes elevated, as activated macrophages are a source of both ACE and 1,25-dihydroxy-cholecalciferol (the active metabolite of vitamin D_3).

Crohn's disease – This is another non-infectious disease in which granulomas are prominent. In Crohn's disease, a chronic inflammatory disease of the ileum and colon, lymphocytes and macrophages accumulate in all types of the bowel. The granulomatous reaction and fibrosis cause stricture of the bowel and penetrating fistulas into other organs. The natures of the antigens or infectious agents initiating and perpetuating this granulomatous reaction are unknown.

Activated T cells, showing restricted T cell receptor repertoire and a TH1-like profile of cytokine production, are responsible for macrophage activation and the release of inflammatory cytokines, such as TNF, reactive oxygen metabolites and nitric oxide. These initiate and maintain the transmural intestinal inflammation. Inhibition of TNF activity with antibody reduces inflammation in patients with Crohn's disease.

The variants of delayed hypersensitivity

delayed reaction	maximal reaction time
contact	48–72 hours
tuberculin	48–72 hours
granulomatous	21–28 days

Figure-1 Contact and tuberculin-type hypersensitivity have a similar time course and are maximal at 48-72 hours. In certain circumstances (e.g. with insoluble antigen) granulomatous reactions also develop at 21-28 days (e.g. skin testing in leprosy).
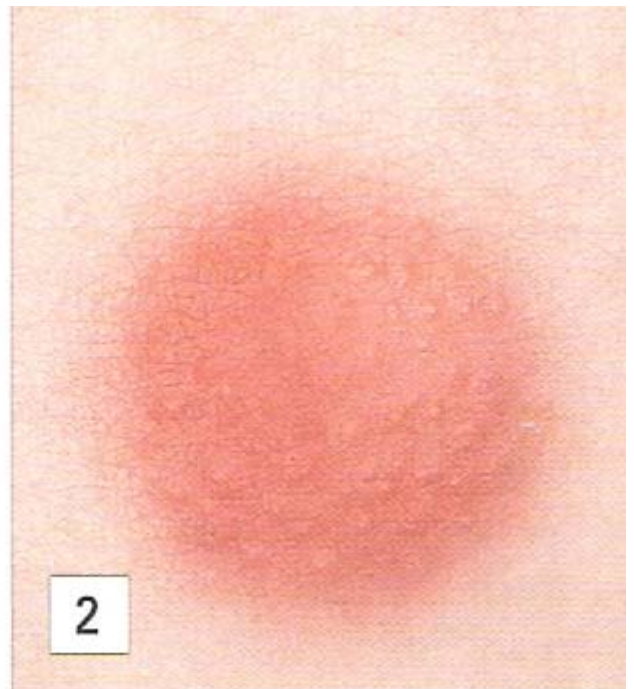
Figure-2 Clinical and patch-test appearances of contact hypersensitivity. (1) The eczematous area at the wrist is due to sensitivity to nickel in the watch-strap buckle. (2) The suspected allergy may be confirmed by applying potential allergens, in the relevant concentrations and vehicles, to the patient's upper back (patch testing). A positive reaction causes a localized area of eczema at the site of the offending allergen, 2-4 days after application.
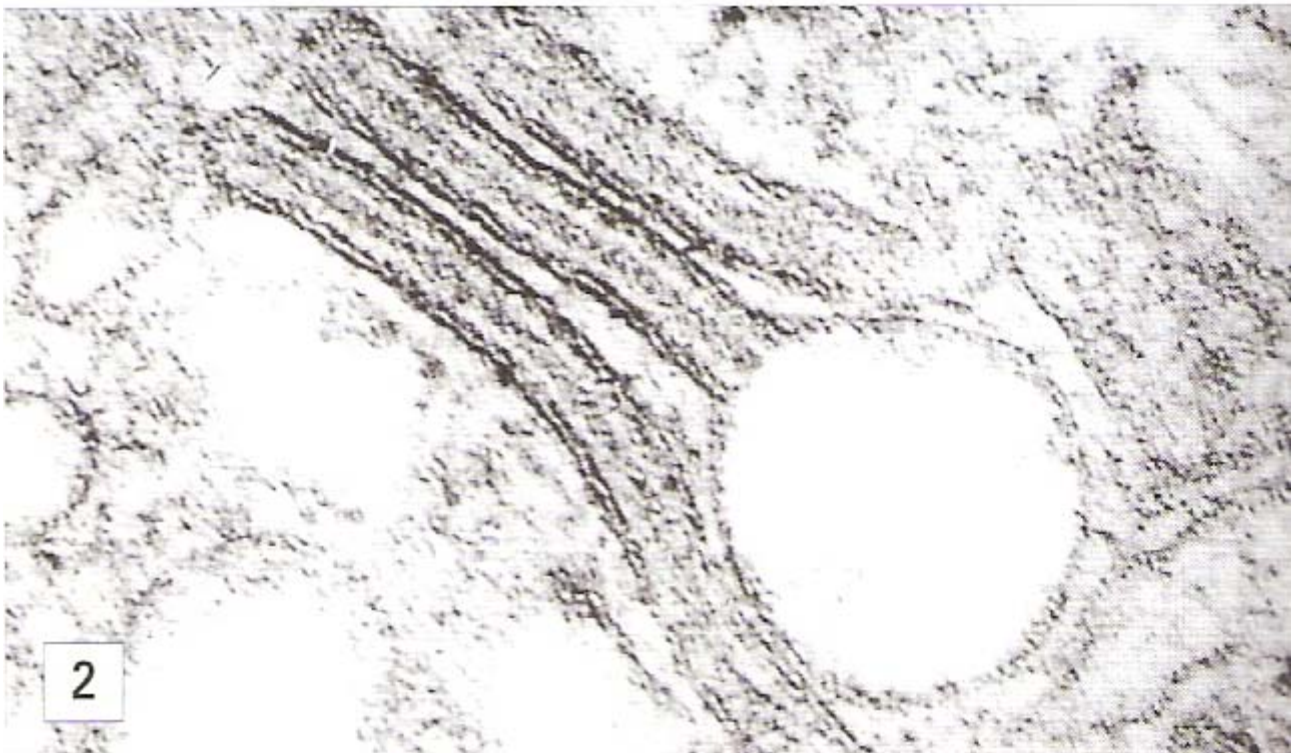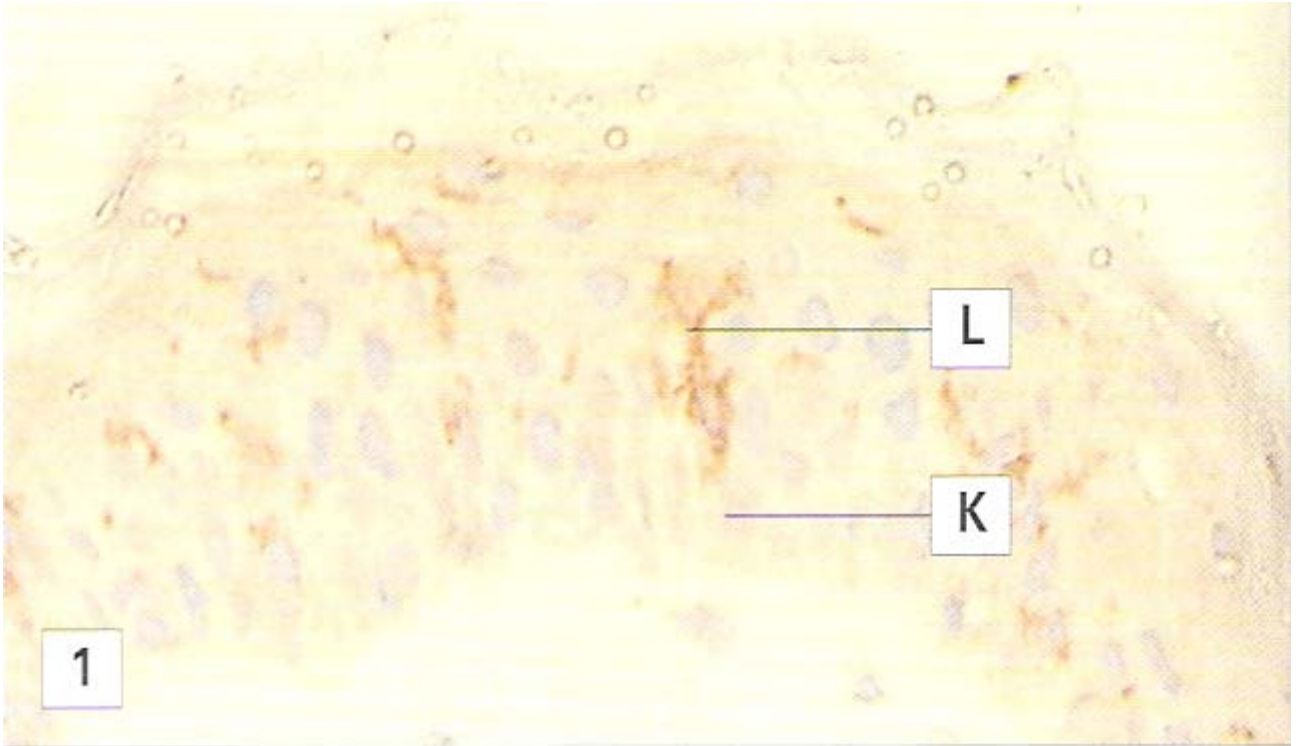
Figure-3 The Langerhans' cell (1) These dendritic cells constitute 3% of all cells in the epidemis. They express a variety of surface markers which allow them to be visualized.

Here they have been revealed in a section of normal skin using a monoclonal antibody which reacts with the CD1antigen (counterstained with Mayer's haemalum). L=Langerhans' cell; K=keratinocyte. x312. (2) Electron micrograph of a Langerhans' cell showing the characteristic 'Birbeck granule'. This organelle is a plate-like structure with a distinct central striation and often has a belb-like extension at one end. x132000.

Sensitization phase of contact hypersensitivity

hapten–carrier
complex

hapten

epidermis

Langerhans' cell

carrier

dermis

lymphatic

lymph vessel

regional
lymph node

cortex
(B-cell area)

macrophage

secondary
follicle

interdigitating
cell

paracortex
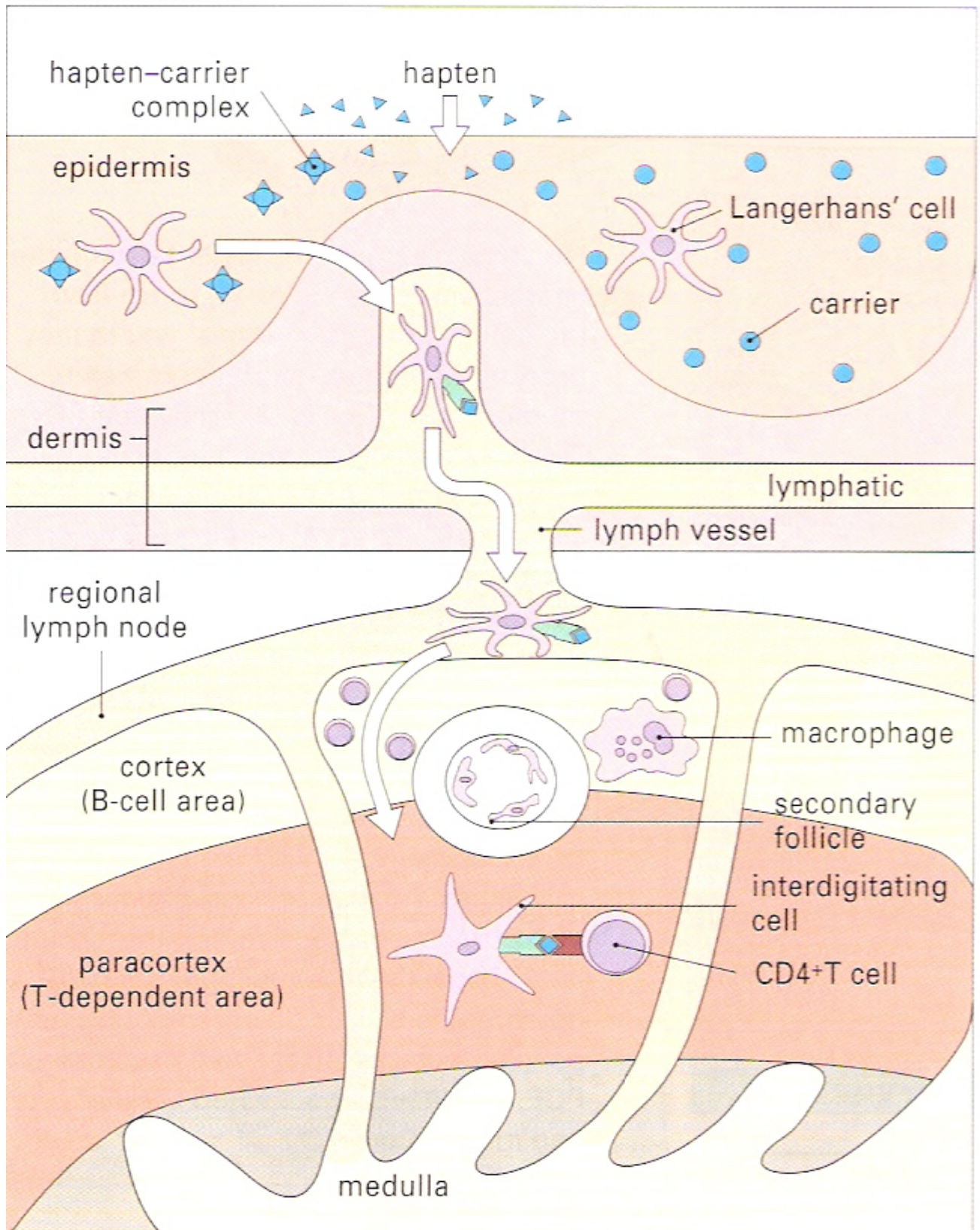(T-dependent area)

CD4+T cell

medulla

Figure-4 The hapten forms a hapten-carrier complex in the epidermis's cells internalize the antigen, undergo maturation, and migrate via afferent lymphatics to the paracortical area of the regional lymph node where peptide/MHC complexes on the surface of the Langerhans' cell can also be directly haptenated. As interdigitating cells, they present antigen to CD4$^+$ T cells.