

Lecture 2: Mechanisms of Immune-Mediated Tissue Injury

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Immune-mediated tissue injury is not a failure of a "different" system from normal immunity — it is the *same* antibody, complement, and T cell effector machinery covered in Lecture 1, acting against an inappropriate target, at an inappropriate magnitude, or for an inappropriate duration.

1. Hypersensitivity Reactions: The Gell and Coombs Classification

Type I hypersensitivity, in the Gell and Coombs classification, is the **immediate hypersensitivity reaction** — mediated by IgE and executed primarily by mast cells and basophils. It is the immunological basis of allergic disease: allergic rhinitis, asthma, food allergy, urticaria, and anaphylaxis. The reaction occurs within minutes of antigen (allergen) exposure in a sensitized individual, distinguishing it from the delayed (Type IV, T-cell-mediated) hypersensitivity that takes 24–72 hours.

The defining feature is that the response is disproportionate to the antigen — allergens are typically innocuous environmental proteins (pollen, dust mite Der p 1, food proteins, venom components) that pose no inherent threat, yet the immune system mounts a Th2-polarized response against them.

Type I: Immediate (IgE-Mediated) Hypersensitivity

Phase One: Sensitization

Before any clinical reaction can occur, the individual must be sensitized — this phase is asymptomatic.

Antigen presentation and Th2 skewing: Allergen is captured by dendritic cells at mucosal surfaces (airway, gut, skin) and presented via MHC class II to naïve CD4+ T cells. In atopic individuals, a combination of genetic predisposition, epithelial barrier signals (TSLP, IL-25, IL-33 released from damaged epithelium), and the nature of the antigen itself favors differentiation into **Th2 cells** rather than Th1 or Th17.

Cytokine-driven class switching: Th2 cells secrete IL-4 and IL-13, which drive B cells to undergo immunoglobulin class switch recombination to produce **IgE** instead of IgG. IL-5 additionally recruits and activates eosinophils, and IL-9 supports mast cell proliferation — these become relevant later.

IgE binds FcεRI: The secreted IgE does not circulate freely for long. It binds with extremely high affinity to **FcεRI (the high-affinity IgE receptor)** on the surface of mast cells (tissue-resident) and basophils (circulating). This "arms" the cell — the mast cell is now coated with allergen-specific IgE, primed but not yet activated. This is the sensitized state.

Phase Two: Effector Reaction Upon Re-exposure

On subsequent exposure, allergen binds the Fab portions of the surface-bound IgE. Because most allergens are multivalent, this causes **cross-linking of adjacent FcεRI-IgE complexes**. Receptor cross-linking is the essential trigger — monovalent binding without aggregation does not activate the cell.

Signal transduction: Cross-linking clusters the FcεRI receptors, activating the receptor-associated Src-family kinase **Lyn**, which phosphorylates ITAMs on the receptor's cytoplasmic tails. This recruits and activates **Syk kinase**, which propagates signaling through the LAT scaffold to activate **PLCγ**, generating IP3 and DAG. IP3 triggers release of intracellular calcium stores, and the resulting calcium flux is the proximal trigger for degranulation. This produces two temporally distinct waves of mediator release.

Mediators and Their Effects

A. Immediate phase (minutes) — Preformed granule contents

Mast cell granules release their contents within minutes via exocytosis:

- **Histamine** — acts on H1 receptors on vascular endothelium and smooth muscle, causing vasodilation, increased vascular permeability (edema), and smooth muscle contraction (bronchoconstriction, gut cramping). H1 activation on sensory nerve endings also produces itch (pruritus).
- **Tryptase and chymase** — proteases that remodel local extracellular matrix and amplify inflammation; tryptase is the standard clinical biomarker used to confirm mast cell activation (e.g., in suspected anaphylaxis).
- **Heparin** — a proteoglycan with local anticoagulant activity, packaged alongside the proteases.

B. Newly synthesized lipid mediators (minutes to ~1 hour)

Membrane phospholipids are cleaved by phospholipase A2 to release **arachidonic acid**, which is metabolized via two enzymatic pathways:

- **Cyclooxygenase pathway → Prostaglandin D2 (PGD2)**: causes bronchoconstriction and vasodilation, and is a potent chemoattractant for Th2 cells and eosinophils.
- **5-Lipoxygenase pathway → Leukotrienes C4, D4, E4 (the "cysteinyl leukotrienes," historically termed slow-reacting substance of anaphylaxis)**: these are substantially more potent than histamine on a molar basis for bronchoconstriction and vascular permeability — this is the pathway targeted by drugs like montelukast.
- **Platelet-activating factor (PAF)**: contributes to bronchoconstriction, vascular permeability, and platelet aggregation, and is an important contributor to severe/fatal anaphylaxis.

C. Late phase (4–8 hours) — Cytokine-driven cellular recruitment

Mast cell activation also triggers transcription of cytokine genes (this is slower than degranulation, since it requires new protein synthesis): **TNF- α , IL-4, IL-5, IL-13**. These, combined with chemokines like eotaxin, recruit and activate a second wave of effector cells — chiefly **eosinophils**, but also basophils and Th2 cells — to the tissue over several hours. This produces the **late-phase reaction**: renewed swelling, redness, and (in the airway) sustained bronchoconstriction and hyperresponsiveness, occurring without additional allergen exposure. Eosinophils release major basic protein and eosinophil cationic protein, which are directly toxic to epithelium and perpetuate tissue damage — this is central to the chronicity of conditions like asthma.

Clinical Consequences by Site and Magnitude

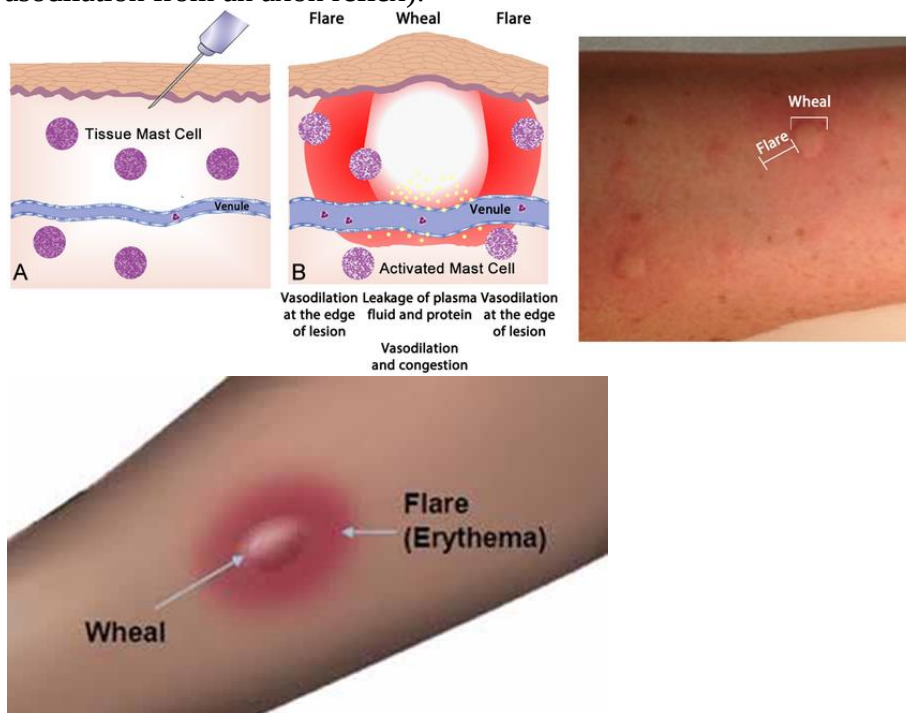
The anatomical location of mast cell activation and the extent of systemic involvement determine the clinical phenotype:

Site/Extent	Consequence
Skin	Urticaria (wheal and flare), angioedema
Nasal/conjunctival mucosa	Allergic rhinitis, allergic conjunctivitis
Lower airway	Asthma — bronchoconstriction, mucus hypersecretion, airway edema
GI tract	Vomiting, diarrhea, cramping (food allergy)
Systemic/vascular	Anaphylaxis — mast cell activation across multiple organ systems simultaneously produces widespread vasodilation and vascular permeability, causing distributive shock, laryngeal edema (airway obstruction), and severe bronchospasm; this is the life-threatening extreme of Type I hypersensitivity

- **Examples across species**: atopic dermatitis, anaphylaxis (insect stings, drug reactions, vaccine reactions), feline asthma, insect bite hypersensitivity, food allergy

Diagnostic correlate: eosinophilic infiltrate on histopathology, elevated serum IgE or allergen-specific IgE, skin test reactivity, mast cell degranulation on cytology. The **wheal-and-flare reaction** used diagnostically in skin prick testing is a direct, small-scale reproduction of this

mechanism: local histamine release causes the wheal (edema from vascular permeability) and flare (vasodilation from an axon reflex).



Allergen exposure → Th2 skewing → IL-4/IL-13-driven IgE class switching → IgE binds FcεRI on mast cells (sensitization) → re-exposure cross-links IgE-FcεRI → Lyn/Syk/PLCγ signaling → calcium flux → **degranulation** (histamine, tryptase) + **lipid mediator synthesis** (leukotrienes, PGD2, PAF) → **late-phase cytokine release** (eosinophil/Th2 recruitment) → clinical manifestation determined by anatomical distribution and magnitude, ranging from localized urticaria to systemic anaphylaxis.

Type II: Antibody-Mediated Cytotoxic Hypersensitivity

Type II hypersensitivity is **antibody-mediated cytotoxicity** — IgG or IgM antibodies bind directly to antigens present on the surface of the body's own cells or fixed within tissue matrix, and the resulting antibody-antigen complex triggers cell or tissue destruction. This is fundamentally different from Type I: there is no IgE, no mast cell, and no requirement for cross-linking of a receptor to trigger degranulation. Instead, the antibody itself is the direct instrument of damage, either by recruiting destructive effector mechanisms or by binding a functional receptor and perturbing its signaling.

The target antigen can be:

- **Intrinsic** — a normal self-antigen mistakenly recognized as foreign (autoimmune), or a foreign antigen adsorbed onto a self-cell (e.g., a drug hapten on an erythrocyte membrane).
- A cell-surface **receptor**, where antibody binding alters function without necessarily destroying the cell — this variant is sometimes separated out as "Type V" but is conventionally taught within Type II.

The Sensitization Step

Unlike Type I, there is no obligatory prior asymptomatic sensitization phase with an "arming" step analogous to IgE-FcεRI loading. Type II reactions arise whenever IgG or IgM antibodies specific for a cell-surface or matrix antigen are present — whether from breakdown of self-tolerance (autoimmunity), alloimmunization (transfusion, pregnancy), or drug-induced neoantigen formation. Antibody production still requires B cell activation, typically with T cell help and germinal center maturation, but the clinically relevant event is what happens once

antibody is bound to the target tissue — there is no equivalent "silent primed" cell waiting for cross-linking.

Effector Mechanisms — How Antibody Binding Causes Damage

Once IgG or IgM coats a target cell or tissue surface, three principal destructive pathways can be engaged, often in combination.

A. Complement-dependent cytotoxicity (CDC)

Antibody bound to the cell surface exposes its Fc region, which is recognized by **C1q**, initiating the classical complement cascade. This proceeds through C3 and C5 convertase formation to generate the **membrane attack complex (MAC, C5b-9)**, which perforates the target cell membrane and causes osmotic lysis. Complement activation also generates the anaphylatoxins **C3a and C5a**, which recruit and activate neutrophils and macrophages, and the opsonin **C3b**, which flags the cell for phagocytosis (see below). This is the dominant mechanism in classic examples like ABO-mismatched transfusion reactions and autoimmune hemolytic anemia.

B. Antibody-dependent cellular cytotoxicity (ADCC) and opsonization/phagocytosis

The Fc portion of antibody bound to the target is recognized by **Fcγ receptors** on effector cells:

- **Macrophages and neutrophils** bearing FcγR engulf and destroy antibody-coated (opsonized) cells — this occurs prominently in the spleen for opsonized red cells (extravascular hemolysis) and at tissue sites for opsonized basement membrane components.
- **NK cells** bearing FcγRIII (CD16) engage antibody-coated targets and release perforin and granzymes, inducing apoptosis without needing antigen-specific T cell recognition — this is the mechanism exploited by therapeutic antibodies like rituximab.

C. Antibody-mediated cellular dysfunction (no cell death)

In a subset of Type II reactions, antibody binds a functional cell-surface receptor and **modulates its signaling** rather than destroying the cell:

- **Stimulating:** anti-TSH receptor antibodies in Graves' disease constitutively activate the receptor, driving unregulated thyroid hormone production.
- **Blocking:** anti-acetylcholine receptor antibodies in myasthenia gravis occupy and downregulate the receptor, and also fix complement, causing loss of the post-synaptic membrane, and impair neuromuscular transmission without necessarily killing the muscle cell.
- **surface or tissue matrix**, triggering one or more effector pathways:
 - **Complement-mediated lysis** (classical pathway → MAC formation)
 - **Opsonization and phagocytosis** (Fc receptor-mediated uptake by macrophages, especially in spleen/liver)
 - **Antibody-dependent cell-mediated cytotoxicity (ADCC)** via NK cells engaging the antibody Fc region
 - In some cases, antibody binds a functional receptor and either blocks or inappropriately stimulates it (receptor-mediated dysfunction, distinguished by some schemes as "Type V" but conventionally grouped under Type II)

"Mediators" in Type II — What Actually Does the Damage

Since Type II is not granule-mediator-driven like Type I, the relevant "mediators" are the components of the effector mechanisms themselves:

Mediator	Origin	Effect
C5b-9 (MAC)	Complement cascade	Direct membrane lysis
C3a, C5a	Complement cleavage	Neutrophil/macrophage chemotaxis and activation; local inflammation
C3b	Complement cleavage	Opsonization → phagocytic clearance

Perforin/granzyme B	NK cells (ADCC)	Induction of target cell apoptosis
Reactive oxygen species, proteases	Activated neutrophils/macrophages	Bystander tissue damage, particularly at fixed tissue antigens (e.g., basement membrane)
The antibody itself (agonist/antagonist)	Autoreactive B cells	Receptor stimulation or blockade, independent of any cytotoxic mediator

A key conceptual point: in receptor-modulating disease (Graves', myasthenia gravis), the antibody is the mediator — there is no downstream cascade required for the core pathology, although complement and inflammation often contribute secondary damage.

5. Clinical Consequences by Target and Mechanism

Target antigen	Disease	Dominant mechanism
RBC surface antigens (Rh, ABO, drug-hapten)	Autoimmune hemolytic anemia, hemolytic transfusion reaction, hemolytic disease of the newborn	Complement lysis + Fc-mediated splenic phagocytosis
Platelet glycoproteins (GPIIb/IIIa)	Drug-induced immune thrombocytopenia	Fc-mediated phagocytosis, complement
Glomerular/alveolar basement membrane (type IV collagen)	Goodpasture syndrome	Complement + neutrophil-mediated tissue damage → glomerulonephritis, pulmonary hemorrhage
Desmosomal proteins (desmoglein)	Pemphigus vulgaris	Direct disruption of keratinocyte adhesion + complement
Acetylcholine receptor	Myasthenia gravis	Receptor blockade/internalization + complement-mediated membrane loss
TSH receptor	Graves' disease	Receptor stimulation (no cell death)
Transplanted graft endothelium	Hyperacute transplant rejection	Pre-formed antibody → complement → vascular thrombosis

Self- or foreign-antigen expressed on a cell surface or fixed tissue → IgG/IgM antibody binds it → **three possible downstream paths**: (1) complement fixation → MAC-mediated lysis, (2) Fc-receptor engagement → phagocytosis or NK-mediated ADCC, or (3) direct receptor agonism/antagonism without cytotoxicity → clinical phenotype determined by which tissue expresses the target antigen and which of these three mechanisms predominates.

- **Key distinguishing feature vs. Type III**: the antigen is an intrinsic component of the target cell/tissue itself, not a soluble circulating antigen forming complexes elsewhere
- **Examples across species**: autoimmune hemolytic anemia (AIHA), immune-mediated thrombocytopenia (IMTP), pemphigus (anti-desmoglein antibodies disrupting keratinocyte adhesion), Goodpasture-type anti-basement membrane disease, neonatal isoerythrolysis (maternal alloantibody against neonatal RBC antigens — important in foals and some other species)
- **Diagnostic correlate**: direct Coombs test (detects antibody/complement bound to RBC surface), immunofluorescence showing antibody deposition along a defined tissue structure (e.g., linear pattern along basement membrane)

Type III: Immune Complex-Mediated Hypersensitivity

Type III hypersensitivity is **immune complex-mediated** disease. Unlike Type II, where antibody binds an antigen that is *already part of* a cell surface or tissue structure, in Type III the antigen is typically **soluble** and circulates freely. Antibody (IgG, occasionally IgM) binds this soluble antigen in the fluid phase, forming **immune complexes**, which then deposit in

tissues — most characteristically in blood vessel walls, joints, and the renal glomerulus — and trigger inflammation at the site of deposition rather than at the site of antigen production. The critical distinction from Type II: damage in Type III is not targeted at a specific cell that expresses the antigen. It is essentially a **bystander phenomenon** — any tissue where complexes happen to lodge becomes collateral damage, which is why Type III disease tends to be systemic and multi-organ rather than confined to one cell lineage.

Formation of Immune Complexes — Why Size Matters

When antibody meets soluble antigen, the size of the resulting complex depends on the ratio of antibody to antigen, and this size is the single most important determinant of pathogenicity.

- **Large complexes** (formed in antibody excess) are efficiently opsonized via C3b and cleared by the mononuclear phagocyte system (Kupffer cells, splenic macrophages) — these are largely harmless.
- **Small complexes** (formed in large antigen excess) tend to remain soluble and are eventually filtered without much tissue engagement.
- **Intermediate-sized complexes** (formed near antigen-antibody equivalence, or under conditions of mild antigen excess) are the pathogenic ones — they are too large to be freely filtered but not efficiently phagocytosed, so they persist in circulation and passively deposit in vessel walls, particularly at sites of high hydrostatic pressure and turbulent flow (glomerular capillaries, joint synovium, choroid plexus, skin venules).

Deposition is favored by increased vascular permeability (itself potentiated by vasoactive amines from platelets and mast cells, which can be triggered by complexes engaging FcγRs and complement receptors on these cells) and by hemodynamic factors that promote complex trapping in high-flow, high-pressure filtration beds.

Effector Mechanism — What Happens After Deposition

Once complexes lodge in a vessel wall or tissue basement membrane, the same Fc- and complement-dependent mechanisms from Type II are engaged — but now directed at the tissue itself rather than a cell surface antigen:

Complement activation: The Fc regions of complexed antibody engage **C1q**, activating the classical pathway. This generates:

- **C3a and C5a (anaphylatoxins)** — these are the central pathogenic mediators of Type III. They increase local vascular permeability and are potent **chemoattractants for neutrophils**.
- **C5b-9 (MAC)** — contributes directly to cell/tissue injury, particularly in the glomerular basement membrane.

Neutrophil recruitment and frustrated phagocytosis: Neutrophils arrive at the deposition site and attempt to phagocytose the complexes via FcγR and complement receptors. Because the complexes are embedded in a fixed tissue structure (basement membrane, vessel wall) rather than presented as a discrete particulate target, the neutrophils cannot internalize them — a phenomenon termed "**frustrated phagocytosis**." In this state, neutrophils release their granule contents extracellularly:

- **Lysosomal proteases** (elastase, collagenase) — digest basement membrane and vessel wall matrix components
- **Reactive oxygen species** — cause direct oxidative damage to endothelium and adjacent tissue

This extracellular degranulation is the proximal cause of tissue injury in Type III — the neutrophil essentially digests the vessel wall in its failed attempt to clear the complex.

Platelet involvement: Complexes can also engage platelets via FcγRIIA, triggering aggregation and microthrombus formation, which contributes to the vasculitic/ischemic component of tissue damage in some presentations.

Mediators and Their Effects — Summary Table

Mediator	Source	Effect
C3a, C5a	Complement classical pathway	Vascular permeability, neutrophil chemotaxis/activation
C5b-9 (MAC)	Complement cascade	Direct membrane/basement membrane injury
Neutrophil elastase, collagenase	Frustrated phagocytosis	Basement membrane and vessel wall degradation
Reactive oxygen species	Activated neutrophils	Oxidative endothelial/tissue damage
Vasoactive amines (histamine, serotonin)	Platelets, mast cells (via FcγR/complement receptor engagement)	Increased vascular permeability, promoting further complex deposition
Microthrombi	Platelet aggregation via FcγRIIA	Local ischemia, contributes to vasculitis

Two Classic Experimental/Clinical Paradigms

Localized reaction — the Arthus reaction: Intradermal injection of antigen into a previously immunized individual (high circulating IgG) causes local immune complex formation at the injection site within hours, producing localized vasculitis with edema, hemorrhage, and neutrophilic infiltration. This is the classic experimental model for Type III and can occur clinically at injection sites of vaccines or insulin in highly sensitized individuals.

Systemic reaction — serum sickness: Classically produced by injection of foreign protein (originally horse-derived antiserum; now more relevant with therapeutic monoclonal antibodies or antivenom). Antigen persists in circulation while antibody titers rise over 1–2 weeks, generating a wave of intermediate-sized complexes that deposit systemically — producing fever, urticaria, arthralgia, lymphadenopathy, and glomerulonephritis roughly 7–10 days after exposure. This time course (delayed relative to exposure, coinciding with the antibody response) is a hallmark that distinguishes it from Type I, which is immediate.

Clinical Consequences by Disease

Disease	Antigen	Primary deposition site
Systemic lupus erythematosus	Nuclear antigens (dsDNA, nucleosomes) — anti-dsDNA/anti-Sm antibodies	Glomerular basement membrane, skin, joints, serosal surfaces
Post-streptococcal glomerulonephritis	Streptococcal antigen-antibody complexes	Glomerulus
Serum sickness	Foreign protein (antiserum, therapeutic antibodies)	Vessels, joints, kidney, skin (systemic)
Polyarteritis nodosa (subset)	Hepatitis B surface antigen complexes	Medium-vessel walls
Rheumatoid arthritis (partly)	IgM anti-IgG (rheumatoid factor) complexes	Synovium
Arthus reaction	Locally injected antigen	Skin/subcutaneous vessels at injection site

Soluble antigen persists in circulation → IgG/IgM binds it in the fluid phase → intermediate-sized immune complexes form (near antibody-antigen equivalence) → complexes evade efficient phagocytic clearance and deposit in vessel walls/basement membranes at high-pressure filtration sites → **complement activation (C3a/C5a)** recruits neutrophils → **frustrated phagocytosis** releases proteases and ROS extracellularly → basement

membrane/vessel wall injury → clinical phenotype (vasculitis, glomerulonephritis, arthritis, serositis) determined by where complexes preferentially deposit.

- **Complex size matters:** intermediate-sized complexes (formed at slight antigen excess) are the most pathogenic because they are large enough to deposit in tissue but too small to be efficiently cleared by the reticuloendothelial system
- Covered in more depth in its own section below given its centrality to systemic immune-mediated disease.

Diagnostic Approach

- **Medical History:** Doctors review your recent exposure to medications, infections, or foreign proteins, along with your symptom timeline.
- **Physical Examination:** Providers look for specific signs like joint swelling, rashes, or local skin lesions depending on whether the reaction is localized (like an Arthus reaction) or systemic (like serum sickness).

Laboratory and Diagnostic Tests

- **Complement Levels:** Blood tests check for low levels of complement proteins (such as **C3 and C4**), which drop when immune complexes consume them during a flare.
- **Antibody and Inflammatory Markers:** Tests may measure general markers like **CRP** (C-reactive protein) and **ESR** (erythrocyte sedimentation rate) for inflammation, or specific antibodies tied to conditions like lupus or rheumatoid arthritis.
- **Tissue Biopsy:** A punch or organ biopsy (such as of the skin or kidney) examined under a microscope can confirm immune complex deposits and blood vessel inflammation.

Type IV: Delayed-Type (Cell-Mediated) Hypersensitivity

Type IV hypersensitivity is the **delayed-type, T-cell-mediated** reaction — the only one of the four classical types that does not depend on antibody. Instead, tissue injury is driven directly by sensitized **T lymphocytes** and the cells they recruit or kill. Because it depends on cellular trafficking and clonal T cell activation rather than a pre-formed antibody-antigen interaction, the reaction takes **24–72 hours** to manifest — hence "delayed-type hypersensitivity" (DTH), in contrast to the minutes-to-hours kinetics of Types I–III.

Type IV is conventionally subdivided into distinct subtypes based on which T cell subset and effector mechanism dominates:

- **IVa (Th1-mediated / Macrophage Activation):**
 - *Mechanism:* Th1 cells release interferon-gamma (IFN-γ) and other cytokines to recruit and activate macrophages, leading to chronic inflammation and tissue induration.
 - *Examples:* Tuberculin skin test (PPD reaction), contact dermatitis (chronic form), and granulomatous hypersensitivity (e.g., tuberculosis).
- **IVb (Th2-mediated / Eosinophil-Rich):**
 - *Mechanism:* Th2 cells secrete IL-5, IL-4, and IL-13, driving eosinophil activation and promoting IgE/IgG4 production. It mimics aspects of Type I immediate hypersensitivity but relies on delayed T-cell activation.
 - *Examples:* Chronic asthma, chronic allergic rhinitis, and maculopapular drug rashes with eosinophilia.
- **IVc (CD8+ Cytotoxic T Cell / Direct Cytotoxicity):**
 - *Mechanism:* CD8+ cytotoxic T lymphocytes (CTLs) directly kill target host cells using perforin, granzyme B, and Fas ligand.
 - *Examples:* Contact dermatitis (acute blistering), Stevens-Johnson syndrome / toxic epidermal necrolysis (TEN), and graft rejection.
- **IVd (Th17-mediated / Neutrophilic):**

- **Mechanism:** Th17 cells release IL-8, IL-17, and GM-CSF, causing a massive recruitment and activation of neutrophils.
- **Examples:** Psoriasis, severe neutrophilic cutaneous reactions, and acute generalized exanthematous pustulosis (AGEP).

This subclassification matters clinically because it explains why different Type IV diseases have very different histological pictures despite all being "T-cell mediated."

Phase One: Sensitization

As with Type I, there is an obligatory priming phase, but the end product is a population of memory/effector T cells rather than antibody.

Antigen presentation: Antigen (often a small reactive molecule — a hapten — or a peptide from an intracellular pathogen) is captured by dendritic cells, processed, and presented on MHC. Peptide-MHC class II presentation to CD4⁺ T cells drives Th1/Th2/Th17 differentiation depending on the local cytokine milieu (IL-12 favors Th1; IL-4 favors Th2; IL-1 β /IL-6/TGF- β /IL-23 favor Th17). Peptide-MHC class I presentation (relevant for viral antigens, intracellular pathogens, and haptens that modify intracellular proteins) primes CD8⁺ T cells toward a cytotoxic phenotype.

Clonal expansion and memory formation: Primed T cells proliferate and a subset persists as long-lived memory T cells. This is the sensitized state — clinically silent, but on re-exposure, these memory cells can be recruited to the tissue and reactivate far faster than a naïve response would allow, producing the reaction within 1–3 days rather than the 1–2 weeks required for de novo priming.

Phase Two: Effector Mechanisms on Re-exposure

A. IVa — Th1/macrophage-mediated (the "classic" DTH reaction)

On re-exposure, antigen-presenting cells in the tissue re-present antigen to circulating memory Th1 cells. Activated Th1 cells secrete **IFN- γ** , which is the dominant mediator of this subtype. IFN- γ :

- Activates local macrophages, upregulating their phagocytic and microbicidal capacity (increased lysosomal enzyme content, reactive oxygen and nitrogen intermediates)
- Upregulates MHC class II and co-stimulatory molecules, amplifying further antigen presentation
- Drives macrophage differentiation into **epithelioid cells**, which can fuse into **multinucleated giant cells** when the antigen cannot be cleared — this is the basis of **granuloma formation**

TNF- α (from both T cells and activated macrophages) upregulates endothelial adhesion molecules, recruiting additional monocytes to the site, sustaining the infiltrate.

B. IVb — Th2-mediated

Memory Th2 cells release **IL-4, IL-5, IL-13** upon re-exposure. IL-5 recruits and activates **eosinophils**, and IL-4/IL-13 sustain the Th2 loop and can also support ongoing local IgE production. This subtype produces a delayed reaction that is nonetheless eosinophil-rich, seen prominently in certain drug hypersensitivity reactions.

C. IVc — CD8⁺ cytotoxic T lymphocyte-mediated

Memory CD8⁺ T cells recognize peptide-MHC class I on the surface of target cells (e.g., keratinocytes displaying a drug-modified self-peptide) and kill them directly via two mechanisms:

- **Perforin/granzyme B** — perforin polymerizes to form pores in the target membrane, allowing granzyme B entry, which activates caspases and triggers apoptosis
- **Fas-FasL interaction** — CTL-expressed FasL engages Fas on the target cell, independently triggering the apoptotic cascade

This is the mechanism responsible for the extensive keratinocyte death seen in severe cutaneous drug reactions.

D. IVd — Th17-mediated

Memory Th17 cells secrete **IL-17 and IL-8 (CXCL8)**, which recruit and activate **neutrophils** rather than macrophages or eosinophils. This produces a neutrophil-rich pattern distinct from the mononuclear infiltrate of classic IVa reactions.

Mediators and Their Effects — Summary Table

Subtype	Key T cell	Principal mediator(s)	Effector cell recruited	Tissue effect
IVa	Th1	IFN- γ , TNF- α	Macrophages	Epithelioid/giant cell transformation, granuloma
IVb	Th2	IL-4, IL-5, IL-13	Eosinophils	Eosinophilic infiltrate, tissue edema
IVc	CD8+ CTL	Perforin, granzyme B, FasL	None (direct killing)	Apoptosis of target cells
IVd	Th17	IL-17, CXCL8	Neutrophils	Neutrophilic infiltrate, pustule formation

Clinical Consequences by Subtype

Subtype	Disease/Reaction	Notes
IVa	Tuberculin (Mantoux) skin test	The prototypical DTH reaction — induration peaking at 48–72h
IVa	Granulomatous disease (TB, sarcoidosis, leprosy tuberculoid form, chronic berylliosis)	Granuloma forms when the antigen (e.g., mycobacterial cell wall) resists clearance, walling it off
IVa	Allergic contact dermatitis (e.g., poison ivy/urushiol, nickel)	Hapten conjugates to skin protein → Th1 sensitization → re-exposure produces eczematous, vesicular dermatitis at 48–72h
IVb	Chronic asthma (T-cell component), certain maculopapular drug eruptions	Overlaps mechanistically with Type I but reflects delayed T-cell-driven eosinophilia
IVc	Stevens-Johnson syndrome / toxic epidermal necrolysis (SJS/TEN)	Massive CTL-mediated keratinocyte apoptosis; life-threatening epidermal detachment
IVc	Type 1 diabetes mellitus (islet β -cell destruction), graft rejection (cellular component)	Direct CTL killing of self or graft tissue
IVd	Acute generalized exanthematous pustulosis (AGEP)	Neutrophil-rich sterile pustular drug eruption

Antigen (hapten, intracellular pathogen peptide, or autoantigen) presented via MHC I or II → CD4+ Th1/Th2/Th17 or CD8+ T cell priming and clonal expansion (sensitization, asymptomatic) → re-exposure reactivates memory T cells locally → **subtype-specific effector program**: IFN- γ -driven macrophage activation/granuloma (IVa), IL-4/5/13-driven eosinophil recruitment (IVb), direct perforin/granzyme/Fas-mediated cytotoxicity (IVc), or IL-17-driven neutrophil recruitment (IVd) → clinical picture determined by which T cell subset dominates and which tissue is targeted, with onset delayed 24–72 hours because the reaction depends on T cell trafficking and reactivation rather than pre-formed antibody.

Contrast with Types I–III: Type IV is the only category with **no antibody, no complement, and no immediate kinetics** — its hallmark is the 24–72 hour delay reflecting the time needed for memory T cells to traffic to tissue, be reactivated by local antigen presentation, and execute an effector program (cytokine-driven recruitment or direct cytotoxicity) rather than triggering a pre-existing mediator cascade.

- **Mechanism: T cell-mediated**, not antibody-mediated; onset delayed 24–72 hours after antigen exposure (hence "delayed-type")
- Two principal sub-mechanisms:
 - **CD4+ Th1-mediated**: antigen-specific Th1 cells release IFN- γ → activates macrophages → macrophage-driven tissue damage; classic granulomatous pattern if antigen persists (e.g., intracellular pathogens, foreign material)
 - **CD8+ CTL-mediated**: direct cytotoxic killing of antigen-bearing target cells via perforin/granzyme and Fas-FasL pathways
- **Examples across species**: tuberculin-type reactions, contact hypersensitivity, granulomatous disease (mycobacterial, fungal), some drug reactions, graft rejection (acute cellular rejection), and a component of many organ-specific autoimmune diseases (e.g., autoimmune thyroiditis has a significant Type IV component alongside antibody-mediated injury)
- **Diagnostic correlate**: lymphocytic/histiocytic infiltrate on histopathology, granuloma formation, positive delayed skin test reactivity, absence of significant antibody or complement deposition on immunofluorescence (helps distinguish from Types II/III)