Week 3:
Tolerance and autoimmunity I:

Tolerance

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Week 3: Tolerance and Autoimmunity (autoimmune disease = AD)

- Tuesday nov 29th:
  - Lecture 3:1: Tolerance

- Wednesday nov 30th:
  - Lecture 3:2: Autoimmunity

- Tuesday dec 6th:
  - summary case 3
Lecture 3:1

• Principles of Tolerance

• How does the central tolerance limit the development of autoreactive T and B cells?

• How does the peripheral tolerance regulate autoreactive cells in circulation?

• What happens when our maintenance of tolerance fail?
  - Allergy (autoimmune disease/disorders, multifactor pathogenesis)
The immunologic dilemma

cells of the immune system are surrounded by a majority of „self“, and only a few „foreign“

how does the immune system „decide“ against what to react?

- self antigens
- tolerance
- reactivity
- harmless foreign antigen
- foreign pathogen
Nobel prize for discovery of acquired immunological tolerance 1960

• **Burnet** introduced the concept of self/non-self discrimination. “if in embryonic life expandable cells from a genetically distinct race are implanted and established, no antibody response should develop against the foreign cell antigen when the animal takes on independent existence.” *Burnet FM, Fenner F. The Production of antibodies, 1949*

• **Medewar** demonstrated that early engraftment (first few days of life) of donor splenocytes rendered a mouse tolerant to future grafts from the donor strain and not third-party strains. → *Acquired lifelong tolerance*

*Note: Recent experiments have shown that adults can also be tolerated under certain circumstances and that neonates can make effective immune responses if the antigen is presented in sufficiently immunogenic form.*
Induction of specific tolerance

Medewar’s neonatal tolerance experiment

**Week 0**
Foetal or newborn mouse (strain A)
Injected with adult cells from strain B

**Week 6**
Graft skin from strain B
And strain C

**Week 7**
Strain B graft is accepted
Strain C graft is rejected

The mouse has AQUIRED specific tolerance to strain B
Immunological tolerance

• Definition:
  -refers to the **specific immunological non-reactivity** (unresponsiveness) to an antigen due to a previous exposure to the same antigen.
  
- most important form of tolerance is **non-reactivity to self antigens**, it is also possible to induce tolerance to non-self antigens. When an antigen induces tolerance, it is termed as **Tolerogens**.

• Significance:
  - All individuals are tolerant of their own antigens (**self-tolerance**); breakdown of self-tolerance results in autoimmunity
Immunological tolerance

• Like immune response, tolerance is **antigen-specific** (unlike "immunosuppression") and like immunological memory, it can exist in T-cells, B cells or both.

• Tolerance to tissue and cell antigens can be **induced** by injection of **hemopoietic(stem) cells** in neonatal or severely immunocompromised animals.

• **Grafting** of allogeneic bone marrow or thymus in early life results in tolerance to the donor type cells and tissues. Such animals are known as **chimeras**.

• **Therapeutic potential**: Inducing tolerance may be exploited to prevent graft rejection, treat autoimmune and allergic diseases, and prevent immune responses in gene therapy and stem cell transplantation.
Central and peripheral tolerance

Central Tolerance:
It occurs during lymphocyte development.
[Thymus or Bone marrow]

- The principal fate of lymphocytes that recognize self-antigens in the generative organs is death (deletion), **BUT:**

- Some B cells may change their specificity (called “receptor editing”)

- Some T cells may differentiate into regulatory (suppressor) T lymphocytes

Peripheral Tolerance:
Occurs after lymphocytes leave the primary organs.
Induction of central tolerance in T cells

**Failing positive Selection**

- **CD4+/CD8+ Double positive**
  - Death by neglect
  - >90%

- **CD4+ or CD8+ Single-positive**

  - **Intermediate reactivity**
    - USEFUL!

  - **Too strong interaction**
    - With pMHC
    - HARMFUL!

  - **2-5%**

- **Self-MHC-restricted, self-tolerant T-cell repertoire**

  - Export to periphery

- **Negative selection**

- **Positive selection**

- **Pre-selection repertoire**

- **DP thymocytes**

Adapted from Palmer E 2009, Nature Rev imm
Consequences of self antigen recognition in thymus

Positive selection involves recognition of self MHC at cortical epithelial cells (cTECs).

Negative selection predominantly in medulla (Medullary epithelial cells, mTECs)

Selection of autoreactive thymocytes on the basis of thymic antigen dose

Although specificity is guided by TCR, the overall avidity of the interactions, which is influenced by the level of expression of self peptide-MHC, has a substantial effect on T cell fate.

Millar DG, Ohashi PS, 2016, Nat imm 17(2)
Deletion of self-reactive T cells in the thymus: how are self antigens expressed in the thymus?

AIRE (autoimmune regulator) is a putative transcription factor that stimulates expression of many self antigens in the medullary epithelial cells of the thymus (mTECs), required for deletion of self-reactive thymocytes.
Distinct and fluctuating patterns of gene co-expression in subsets of mTECs controlled by AIRE

- Aire-expressing cells are densely packed in medullary regions
- each TRA is only expressed by a minor fraction (1-3%) of mTECs at any given time (ca.100-300 TRA co-expressed)

The APS-1 syndrome: the importance of central mechanisms for maintenance of tolerance against tissue restricted antigens

autoimmune polyglandular syndrome I
- also designated as APECED: (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy)
  • monogenic autoimmune disease
  • Defect in AIRE → decreased expression of self-Ag in thymus → defective negative selection of self-reactive T cells
  • autosomal recessive inheritance (X-linked)

• classical triad of symptoms:
  - adrenal insufficiency (Addison's disease)
  - hypoparathyroidism
  - generalised candidiasis of mucous membranes
  - further endocrine organs may be involved

Chronic mucocutaneous candidiasis in two siblings. Thrush (A) and ungual candidiasis (B) in case 1 at 11 yr of age. Ungual candidiasis (C) in case 2 at 9 yr of age.

Induction of central tolerance in B cells

- Binding to self molecules in bone marrow can lead to the death or inactivation of immature B cells
B-cells Pass Through Several Developmental Checkpoints

- B-cell development shows similar features to T-cell development, but takes place largely in the bone marrow.
- Checkpoints in B-cell development include:
  - Successful expression of Igα and Igβ in late pro-B cells
  - Successful rearrangement at the heavy chain locus in pre B-cells (checkpoint 1)
  - Successful rearrangement at the light chain locus and receptor editing (checkpoint 2)
Why IgD is Important To Make A B-cell Mature?

• Expression of IgD is an important checkpoint in terms of eliminating self reactive B-cells.
• Normally in a mature B-cell, when Antigen binds to mIg of that B-cell, it turns the B-cell on.
• However, before IgD is expressed, if antigen (Self) binds to mIg then the B-cell gets turned off. Further development of that B-cell does not occur, but light chain rearrangement can continue
Receptor editing in central B-cell selection.

- Immature B cells reacting with low to high avidity self-Ag undergo receptor editing
- Reaction with low avidity self-Ag: further differentiation and migration into spleen as anergic or ignorant B cells
- Clonal deletion: frequency unknown
- B cell encountering self-Ag in periphery are represented undergoing peripheral deletion

Experimental setup that tested the relative contribution of receptor editing and clonal deletion to central tolerance of developing 3-83Ig⁺ B cells (Halverson et al. 2004)
Autoreactive and nonautoreactive immature B cells undergo different fates during bone marrow selection.

**Autoreactive Immature B-cell**
- Cytokine receptor
- Ag-mediated BCR signal + cytokine (IL-7?) receptor signal
- Receptor editing with limited cell survival

**Nonautoreactive Immature B cell**
- Tonic BCR signal + BAFF receptor signal
- Differentiation and selection into periphery

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B- Cell Tolerance to Self Antigens

- High affinity IgG production is T cell dependent
- Lack of T cell leads to non-self reactivity by B cells (split tolerance)
- Self reactive B cells either:
  a) Edit their receptors to become non-reactive
  b) Die by the process of apoptosis

- In peripheral B cell tolerance, self reactive cells are removed by negative selection in the spleen in a process that is similar to T cell removal in the thymus.
Central tolerance

• Central tolerance mechanisms regulate self-reactivity in both T and B cells. It is a fundamental process that initially occurs in the thymus (T cells) or bone marrow (B cells).

• **Positive selection** tests for the potential usefulness of the antigen-receptor, whereas **negative selection** removes self-reactive cells from the lymphocyte repertoire.

• Strongly autoreactive CD4 T cells give rise to a subpopulation of self-Ag reactive FoxP3 “natural” Treg cells that suppress autoimmune responses after exiting the thymus.

• Positive selection also coordinates choice of co-receptor expression.

• the autoimmune regulator AIRE drives ectopic expression of many tissue-restricted antigens by mTECs, thus allowing for negative selection against TCRs that could react with these antigens
But there are loads of T cells that recognise self in healthy people

- **GAD** = glutamic acid decarboxylase, expressed in beta-cells and CNS
- Tetramer = MHC class II tetramer with GAD peptide, binds GAD specific T cells
- Danke *et al.* JI 2004
The autoreactive cells are there, but don’t do anything unless provoked

Tg mice carrying TCR specific for LCMV gp bred to tg mice bearing LCMV gp in beta cells
→mice do not develop diabetes unless infected with LCMV
→self-reactive T cells may remain Unresponsive unless provoked

Diabetes is mediated by CD8+ T cells

Ohashi et al. (1991) Cell 65:305
Immunizing healthy mice with autoantigens can brake tolerance

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Disease (human disease model)</th>
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</thead>
<tbody>
<tr>
<td>myelin basic protein</td>
<td>experimental allergic encephalomyelitis (MS)</td>
</tr>
<tr>
<td>proteolipid peptide</td>
<td>“</td>
</tr>
<tr>
<td>myelin oligodendrocyte glycoprotein (MOG)</td>
<td>“</td>
</tr>
<tr>
<td>collagen</td>
<td>experimental arthritis (rheumatoid arthritis)</td>
</tr>
<tr>
<td>thyroid peroxidase</td>
<td>experimental thyroiditis (Graves disease)</td>
</tr>
<tr>
<td>retinal S antigen</td>
<td>experimental uveitis (uveitis)</td>
</tr>
<tr>
<td>acetyl choline receptor</td>
<td>experimental myasthenia gravis (myasthenia gravis)</td>
</tr>
</tbody>
</table>

These antigens are administered in Complete Freund's adjuvant usually with a particular strain of dead mycobacteria H37RA: this gives potent dendritic cell activation. Mouse or rat strain used is important.

By injecting healthy mice with some self-antigen, such as myelin basic protein in adjuvant, quiescent autoreactive T cells that have escaped deletion are primed and cause autoimmune attack on tissues expressing these proteins.
overview peripheral post thymic mechanisms of tolerance:

Thymus

Deletion

Escape

St. Clair EW et al 2007
Ignorance

The self-reactive lymphocyte are present in the periphery, but are unaware of the presence of their auto-antigen, for several reasons:

1. Antigen may simply be present in too low concentration. (threshold for receptor occupancy required to trigger a response)
2. Some antigens are sequestered from the immune system in locations which are not freely exposed to surveillance. = immunologically privileged sites.
   - eye, CNS, testis, placenta and fetus
   - defects in privilege: eg. Sympathetic ophthalmia
3. Control of T-cell trafficking to tissues
   - naive cells recirculate through secondary lymphatic organs and bloodstream, but do not enter into tissues under normal conditions
Anergy
Specific functional unresponsiveness

- TCR ligation in the absence of Costimulation (e.g., absence of infection) leads to inability to express effector functions like cytokine secretion, and makes the cell unresponsive to further stimulation.

- Control of the expression of the costimulatory molecules CD80 and CD86 (B7) is a major mechanism of peripheral Tolerance.

- B cell anergy: lack of T-cell help.
Extrathymic AIRE expressing cells (eTAC) induce anergy in CD4+ T cells

- BDC specific CD4+T cells
- AdBDC transgenic
- Aire driven expression of islet antigen BDC (Chromogranin A)
- BDC specific CD4+T cell proliferation and anergy
Deletion (Activation induced cell-death)

- Activated T- and B-cells upregulate Fas and undergo Fas-FasL mediated apoptosis (AICD)

- Defects in Deletion: Deficiency in the Fas-FasL pathway
  - Mutation in Fas gene → Autoimmune lymphoproliferative disorder (ALPS), systemic inflammation and autoimmunity

- Fas\(^{-/-}\) and FasL\(^{-/-}\) mice develop systemic autoimmunity

- Autoreactive mature CD8+ T cells deleted by extra-thymic Aire-expressing cells (eTACS), (similar to CD8+ thymocyte deletion by mTECs in thymus) ...Gardner JM Science 2008
ALPS Patient 2: An unusual mutation

- Healthy female - at 18 months developed cervical adenopathy.
- Biopsy showed ‘reactive hyperplasia’ Pt developed anemia with hypersplenism, hematuria, proteinuria and renal insufficiency due to mesangial glomerulonephritis, then primary biliary infiltration.
- Evaluation at NIH: lymphadenopathy persists, ANA (+) 1:320, CD4-CD8- cells 25% of T cells, increased B cells; Fas surface expression is normal
Heterozygous Fas splice mutation resulting in loss of exons 3, 4 (AA 52-96)
Suppression
identification of cell populations with suppressive ("regulatory") features

stimulation of naïve T cells over several days with anti-CD3 antibodies induces proliferation

cocultivation of anti-CD3 stimulated naïve T cells with
- CD25- cells (green population)
- CD25+ cells (blue population)
→ A population of CD4+/CD25+ T cells has a dominant suppressive function in vitro

From Hori S et al. Science 2003
**CD4+/25+ nTregs have a suppressive function *in vivo***

- Adoptive transfer of naive T cells into an *immunodeficient* host (lacking T cells of its own) causes autoimmune colitis in the host (blue and red lines).

- Colitis is prevented by co-transfer of purified CD4+/CD25+ regulatory T cells (green line).

- Normal mice contain sufficient autoreactive peripheral T cells to induce autoimmune disease in a host, if they are not restrained.

- Restraint of autoreactive autoimmunity is performed by the population of regulatory T cells.

*Powrie F. Immunol Rev (2005)*
The IPEX syndrome

- immune dysfunction
- polydendocrinopathy
- enteropathy
- X-linked
The IPEX syndrome

- usually lethal within the first few years of life
- multiple autoimmune phenomena, mainly affecting endocrine glands

lymphocytic infiltrate of the skin of a patient with IPEX syndrome
the transcription factor **FOXP3** is the genetic defect of the IPEX syndrome
The *scurfy* mouse: a natural animal model of the IPEX syndrome

- the *scurfy* mouse has multiple autoimmune phenomena reminiscent of the IPEX syndrome

- the gene defect of the *scurfy* mouse was mapped to the *Foxp3* gene

- deletion of this gene causes the same phenotype

- Knockin of a functional copy of the *Foxp3*-Gene in those mice rescues the phenotype
**FOXP3 controls the development of a population of „regulatory“ T cells**

- Foxp3+ cells are characterized by high expression of the alpha chain of the IL-2 receptor (CD25)
- this population is missing in patients with IPEX syndrome
- this population is designated as CD4+/CD25+/FoxP3+ regulatory T cells (Tregs)
How do Tregs exert their suppressive effects?

- many possible mechanisms:
  - production of suppressive cytokines (e.g. IL-10, IL-35, TGFβ)
  - sequestration of IL-2 from effector cells
    - high expression of CD25
  - production of adenosine from extracellular ATP
    - via CD39 (ATPase) and CD73 (nucleotidase)
    - adenosine acts on suppressive A2A receptors present on activated T cells
  - expression of suppressive cell surface markers (e.g. CTLA4)
  - Direct killing (granzyme)

- in vitro evidence for all of these mechanisms
- all may be important in distinct situations
How do Tregs exert their suppressive effects?

One important mechanism is the negative regulation of dendritic cell maturation.

- In the absence of Tregs, effector T cells may exert an "adjuvant effect" on DCs, causing their maturation and facilitating the activation of autoreactive T cells.
- This is blocked by Tregs, keeping DCs in an immature (tolerogenic) state.
How do Tregs exert their suppressive effects?

- The expression of CTLA-4 may be a "core" mechanism of Treg function.
- Selective deletion of CTLA-4 expression in Tregs induces a similar phenotype as total deletion of Tregs.
- Expression of CTLA-4 by Tregs may be a master mechanism to suppress maturation of DCs.

Figure 2: CTLA-4 may be a core mechanism through which T_{reg} cells control APC function. CTLA-4 on T_{reg} cells can trigger signaling through CD80 and/or CD86 on APCs. In addition to direct or indirect modulation of CD80 and CD86 expression, these signals can activate indoleamine-2,3-dioxygenase (IDO), which generates the immunosuppressive mediator kynurenin. These signals can also promote nuclear localization of Foxo transcription factors, which suppress expression of genes encoding IL-6 and tumor necrosis factor P, phosphorylation.
Patients with MS have reduced numbers of CD25+/CD39+ T Cells

Different populations of Tregs

- **Thymus**
  - **nTregs** (natural Tregs)
  - **iTregs** (Induced Treg)

- **Treg** (natural Tregs) with TGFβ, IL-10
- **T naive** (naive T cells) with TGFβ
- **APC** (Antigen Presenting Cells)
- **Thymus-zelle**

Induced Treg (iTregs)
Negative regulation (CTLA-4)

- **Defects in negative regulation**: CTLA-4⁻/⁻ mice die before weaning from multiorgan inflammation

- Polymorphisms in CTLA-4 have been reported in T1D, MS, SLE etc
peripheral tolerance

- central tolerance is important, but leaky:
  - weakly self-reactively lymphocytes are not removed by central tolerance as deletion of those would impose too great limitation on the immune repertoire, resulting in impaired immune responses to pathogens
  - instead, weakly self-reactive cells are suppressed only if they are activated in the periphery (inhibition by Treg)

- CD4+/CD25+ natural Tregs are the paradigm of dominant peripheral tolerance. Deletion of these cells results in multiple and severe autoimmune phenomena

- Immune responses have natural tendency to be self-limited
  - intrinsic programs in activated lymphocytes make them prone to apoptosis
  - activated lymphocytes also acquire sensitivity to external apoptosis-inducing signals (Fas)

- among many other possible mechanisms of action, control of DC maturation by CTLA-4 expressed on Tregs may be a master mechanism by which nTregs exert their suppressive effects.
Factors that promote tolerance to an antigen:

- High doses of antigen
- Long term persistence of antigen in the host
- Intravenous or oral introduction
- Absence of adjuvants (compounds that enhance the immune response to an antigen)
- Low levels of costimulation
- Presentation of antigen by immature, unactivated or actively tolerising antigen presenting cells
Can tolerance be induced artificially?

- Oral tolerance
- Administration of antigen with insufficient adjuvant
- Presentation of antigen by actively tolerising antigen presenting cells
Oral tolerance

• Default response to oral administration of a protein Ag is the development of a phenomenon known as oral tolerance

• Form of peripheral tolerance that renders systemic and mucosal immune system relatively unresponsive to same Ag

• Various mechanisms: anergy, deletion of Ag-specific T cells, generation of iTregs induced in MLN to become gut-homing Ag-specific Foxp3+ Tregs via production of retinoic acid (RA) and TGFβ by migratory DCs (CD103+)
Tolerance to antigens can be experimentally generated by oral administration

- Mice are fed for 2 weeks with 25mg of either ovalbumin, or control protein.
- 7 days later mice are immunized s.c. with ovalbumin + adjuvant.
- After 2 weeks serum Ab’s and T cell function are measured.

→ mice fed with ovalbumin have a lower Ovalbumin specific systemic immune response than those fed the control protein.

Janeway, 9th ed., fig.12.19
Myloral, phase III clinical trial in multiple sclerosis

- Bovine myelin (or vehicle) in tablet form
- 300 mg per day for 2 years
- Recent onset relapsing remitting patients, both male and female, both HLA-DR2- and DR2+
Oral administration of Myelin induces Antigen-specific TGF-β1 secreting T cells in patients with MS

No difference between groups
• lesser than normal progression in the placebo group
→ although mucosal tolerance can be used to avoid inflammatory diseases in animal models of T1D, arthritis and encephalomyelitis, clinical trials in humans have been less successful
How can tolerance be induced artificially?

- Oral tolerance
- Administration of antigen with insufficient adjuvant
- Presentation of antigen by actively tolerising antigen presenting cells
- Cytotoxic drugs
Administration of adjuvant free Glutamic acid decarboxylase peptide (GAD65)

- even in the presence of established Th1 responses, it is possible to induce autoantigen-specific anti-inflammatory Th2 responses.
- Immune deviation of T-cell responses to the beta-cell autoantigen glutamate decarboxylase (GAD65), induced an active form of self-tolerance that was associated with an inhibition of disease progression in prediabetic mice.

Tian et al. Nature 1996
GAD65 treatment in recent onset T1D patients - no clinical effect

Three regimens were administered:
- subcutaneous injections of 20 μg of GAD-alum on days 1, 30, 90, and 270 (four-dose regimen)
- subcutaneous injections of GAD-alum on days 1 and 30 and 2x placebo (two-dose regimen)
- injections of placebo on days 1, 30, 90, and 270.

→ The stimulated C-peptide level declined to a similar degree in all study groups
→ The use of GAD-alum as compared with placebo did not affect the insulin dose, glycated hemoglobin level, or hypoglycemia rate.

Ludvigsson et al. NEJM 2012
How can tolerance be induced artificially?

- Oral tolerance
- Administration of antigen with insufficient adjuvant
- Presentation of antigen by actively tolerising antigen presenting cells
Tolerogenic dendritic cells (tolDC)

- Attempting to skew immune responses away from inflammation by injecting tolerogenic DC pulsed with antigen

DCs secrete many factors that are known to induce tolerance and Treg generation:
- TGFβ and IL-10 mediates Treg differentiation
- DC-derived IL-27 induces Treg generation by increasing IL-10 expression and repressing IL-1β and IL-23 production.
- DC are important source of retinoic acid (RA), involved in the generation of Tregs and inhibition of Th17 cells.
- IDO is a strong inducer of T cell anergy, apoptosis and Treg differentiation.
- IDO may also induce TGFβ expression

Waisman A et al., Semin Immunopathol 2016
Therapeutic effect of tolerogenic dendritic cells in established collagen-induced arthritis is associated with a reduction in Th17 responses.

Collagen II + adjuvant → toIDC

Experimental Rheumatoid Arthritis

Collagen II

Adjuvant

ToIDC

mDC

HBSS

HBSS pulsed unpulsed ToIDC

HBSS

ToIDC 1x10^6

ToIDC 2.5x10^6

HBSS

ToIDC dose (x10^6)

Arthritis & Rheumatism

Volume 62, Issue 12, pages 3656-3665, 30 NOV 2010 DOI: 10.1002/art.27756

http://onlinelibrary.wiley.com/doi/10.1002/art.27756/full#fig3

Stoop et al. Arthritis and Rheumatism 2010
AutoDECRA, autologous tolerogenic DC injected into the inflamed joint

- No clinical benefit
- Antigen?
- Timing?
- Place?
What happens when our maintenance of tolerance fail?

• Harmless antigens are mistakenly considered to be dangerous → hypersensitivity

• Self-antigens are mistakenly considered foreign → autoimmunity
Allergy and allergic diseases
Definitions:

- In susceptible individuals, immune responses to otherwise innocuous Ag can produce allergic reactions upon reexposure to the same Ag.
- Allergic reactions can occur in response to harmless “environmental” Ag such as pollen, food and drugs.
- *Historical* classification by Gell and Coombs into type I-IV hypersensitivity
  - type I: immediate-type allergic reactions mediated by IgE
  - type II: driven by antigen-specific IgG, effector mechanism: complement
  - type III: driven by antigen-specific IgG, FcR-bearing cellular effectors
  - type IV: driven by cellular effectors (lymphocytes and myeloid cell types)

*But, we know today:* most host immune responses involve both humoral and cellular response →Definitions: type I (Th1), type II (Th2), type III (Th17) immune responses
Many types of Allergens can cause allergic sensitization

- Most airborne allergens are small, highly soluble proteins carried on dry particles such as pollen grains or mite feces.
- On contact with mucus-covered epithelia (eyes, nose airways) soluble allergen is eluted from particle, diffuses into mucosa and gets picked up by DCs and provoke sensitization.
- No common characteristics of all allergens have been found.

- Maximum exposure of person to allergen leading to sensitization:
  - pollen allergens in ragweed: 1µg per year (low dose sensitization)
  - bee venom 20-75µg per individual bee sting
  - food allergy: many gramms of allergenic food over prolonged time
  - penicillin-type drugs: i.m. or i.v. injections, 1-2 gramm per injection
Proteases are common sensitizing agents

- E.g. cysteine protease "Der p 1" in dust mite feces -cleaves occludin, a protein component of tight junctions in airway mucosa, destroying integrity between epithelial cells and gaining excess to subepithelial APCs

- **Netherton's syndrome**: high levels of IgE and multiple allergies -loss of function mutation in a protease inhibitor SPINK5 (encoding for lymphoepithelial kazal type-related inhibitor, LEKTI)
IgE and IgE mediated allergic diseases

- Most serum IgE in developed nations is directed against an innocuous antigen - the allergen -
- Most common form of allergic response are to airborne allergens causing symptoms affecting Eg.nasal passages, eyes, lower airways and lungs
- Systemic Reactions (anaphylaxis) occurring at locations distant form site of entry of challenging Ag

<table>
<thead>
<tr>
<th>Reaction or disease</th>
<th>Common stimuli</th>
<th>Route of entry</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic anaphylaxis</td>
<td>Drugs, venoms, Food, e.g. peanuts, serum</td>
<td>i.v. (either directly or following absorption into the blood after oral intake)</td>
<td>Edema, increased vascular permeability, Laryngeal edema, circulatory collapse, death</td>
</tr>
<tr>
<td>Acute urticaria (wheal-and-flare)</td>
<td>Post-viral, animal hair, bee stings, allergy testing</td>
<td>Through skin systemic</td>
<td>Local increase in blood flow and vascular permeability, Edema</td>
</tr>
<tr>
<td>Seasonal rhinoconjunctivitis (hay fever)</td>
<td>Pollens (ragweed, trees, grasses) Dust mite feces</td>
<td>Contact with conjunctiva of eye and nasal mucosa</td>
<td>Edema of conjunctiva and nasal mucosa, sneezing</td>
</tr>
<tr>
<td>Asthma</td>
<td>Dander (cat) Pollens Dust-mite feces</td>
<td>Inhalation leading to contact with mucosal lining of lower airways</td>
<td>Bronchial constriction Increased mucus production, airway inflammation, bronchial hyperreactivity</td>
</tr>
<tr>
<td>Food Allergy</td>
<td>Peanuts, tree nuts, shellfish, fish, milk, eggs, soy, wheat</td>
<td>oral</td>
<td>Vomiting, diarrhea, pruritus (itching), Urticaria (hives), anaphylaxis (rarely)</td>
</tr>
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</table>
**Sensitization to an inhaled allergen**

- **Immediate hypersensitivity reactions**: triggered when allergens cross-link pre-formed IgE bound to the high-affinity receptor FcεRI on mast cells
- **Typical type-2 immune reactions**, 2 main groups of signals:
  1. differentiation of naive T cells to a Th2 response (exposure to IL-4, IL-5, IL-9 and IL-13 favors development of Th2 cells)
  2. Th2 cytokines (IL-4, IL-13)+co-stimulatory signals stimulating B cells to switch to production of IgE
Cellular culprits of allergy: Mast cells

- Once activated, induction of inflammatory reactions by secreting pharmacological mediators such as histamine, stored in preformed granules
- Consequence of mast cell activation depends on dose of Ag and route of entry
- Symptoms range from swollen eyes to life-threatening circulatory collapse
Ag binding to IgE on basophils or mast cells leads to amplification of IgE production

- IgE production can be amplified by these cells, because, upon activation, they produce IL-4 and express CD40 ligand
Tendency of IgE overproduction is influenced by both genetic and environmental factors

- A predisposition to become IgE-sensitized to environmental allergens is called atopy.

- **Hygiene hypothesis**: exposure to some infections and to common environmental microorganisms in infancy and childhood drives the immune system toward a general state of non-atopy.
Effector mechanisms in IgE mediated allergic reactions

- Allergic response to innocuous Ag reflects pathophysiological aspects of a defensive immune response whose physiological role is to protect against helmith parasites.
- Triggered by the binding of Ag to IgE Abs bound to the high-affinity IgE receptor FcεRI on mast cells and basophils.
- Mast cells are strategically distributed beneath the mucosal surfaces of the body and in connective tissue.
- Release of large amounts of inflammatory mediators by mast cells results in inflammation and tissue damage.
  - Early events: short-lived mediators (histamine), rapid onset.
  - Later events: involve leukotrienes, cytokines, chemokines that recruit and activate eosinophils, basophils, other leukocytes.
  - Can evolve to chronic inflammation (effector T cells, eosinophils).
  - E.g. chronic allergic asthma.
Chronic allergic asthma

- IgE-mediated respiratory disease
- Triggered by allergen-induced activation of submucosal mast cells in lower airways
- Can lead within seconds to broncial constriction and increased secretion into airways of fluid and mucus
- Has been associated with inhalation of * Alternaria * spores.
- Chronic inflammation of airways (increased numbers of pathologic lymphocytes, eosinophils, neutrophils, basophils and other leukocytes cause airway hyperreactivity and remodeling → thickening airway walls, fibrotic remodeling lead to a permanent narrowing of the airways.

*Fig. 14.13 Histologic evidence of chronic inflammation in the airways of an asthmatic patient.* Panel a shows a section through a bronchus of a patient who died of asthma; there is almost total occlusion of the airway by a mucus plug. In panel b, a close-up view of the bronchial wall shows injury to the epithelium lining the bronchus, accompanied by a dense inflammatory infiltrate. Although not discernable at this magnification, the infiltrate includes eosinophils, neutrophils, and lymphocytes. Photographs courtesy of T. Krausz.
Chronic allergic asthma

- “endotypes”: common allergic asthma (eosinophil-driven, Th2), exercise-induced asthma, neutrophil-predominant asthma (Th17), steroid-resistant asthma

- each individual patient has its own endotype: result of the specific condition under which individual was sensitized to allergen + specific predisposition based on inherited genetic factors + environmentally determined epigenetic factors

- Fundamental driver of allergic response: pathologically activated Th2 cells, eosinophils and basophils are prominent in inflammatory infiltrates in lungs

Lambrecht BN, Nature Immunology 16, 45-56 (2015)
### Treatments for allergic disease

<table>
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<tr>
<th>Target</th>
<th>Mechanism of treatment</th>
<th>Specific approach</th>
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<td><strong>In clinical use</strong></td>
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<td>Mediator action</td>
<td>Inhibit effects of mediators on specific receptors</td>
<td>Antihistamines, β-agonists</td>
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<td>Inhibit synthesis of specific mediators</td>
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<td>(T_h^2) response</td>
<td>Induction of regulatory T cells</td>
<td>Desensitization therapy by injections of specific antigen</td>
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<td>IgE binding to mast cell</td>
<td>Bind to IgE Fc region and prevent IgE binding to Fc receptors on mast cells</td>
<td>Anti-IgE antibodies (omalizumab)</td>
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<tr>
<td>(T_h^2) activation</td>
<td>Induction of regulatory T cells</td>
<td>Injection of specific antigen peptides</td>
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<td>Administration of cytokines, e.g., IFN-γ, IL-10, IL-12, TGF-β</td>
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<td>Use of adjuvants such as CpG oligodeoxynucleotides to stimulate (T_h^1) response</td>
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<td>Activation of B cell to produce IgE</td>
<td>Block co-stimulation</td>
<td>Inhibit CD40L</td>
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<td>Inhibit (T_h^2) cytokines</td>
<td>Inhibit IL-4 or IL-13</td>
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<td>Mast-cell activation</td>
<td>Inhibit effects of IgE binding to mast cell</td>
<td>Blockade of IgE receptor</td>
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<td>Eosinophil-dependent inflammation</td>
<td>Block cytokine and chemokine receptors that mediate eosinophil recruitment and activation</td>
<td>Inhibit IL-5</td>
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<td>Block CCR3</td>
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Non-IgE-mediated allergic diseases

- Reflects normal immune mechanisms that are inappropriately directed against innocuous Ag or inflammatory stimuli

- **Immediate-type reactions:**
  - binding of specific IgG Abs to allergen-modified cell surfaces
    - e.g. drug-induced hemolytic anemia
  - formation of immune complexes of Ab bound to poorly catabolized Ag
    - e.g. serum sickness

- **Delayed-type reactions:**
  - Th1-mediated hypersensitivity reaction in the skin provoked by *Mycobacterium tuberculosis*
    - used to diagnose previous exposure
  - recognition and destruction by cytotoxic T cells of skin cells modified by a plant molecule
    (allergic reaction to poison ivy, develops over 1-10 days)
Delayed type hypersensitivity

Contact-sensitizing agent penetrates the skin and binds to self proteins, which are taken up by Langerhans cells.

Langerhans cells present self peptides haptenated with the contact-sensitizing agent to Th1 cells, which secrete IFN-γ and other cytokines.

Activated keratinocytes secrete cytokines such as IL-1 and TNF-α and chemokines such as CXCL8, CXCL11, and CXCL9.

The products of keratinocytes and Th1 cells activate macrophages to secrete mediators of inflammation.
Summary Allergy

• Most allergic reactions involve the production of IgE Ab’s against common environmental allergens

• Some people are intrinsically prone to making IgE Ab’s against many allergens → atopic

• IgE production is driven by antigen-specific Th2 cells; the response is skewed toward Th2 by an array of chemokines and cytokines that engage specific signaling pathways (incl. signals activating ILC2s in submucosal tissues at sites of Ag entry)

• Produced IgE binds to high affinity IgE receptor on mast cells and basophils

• Specific effector T cells, mast cells, eosinophils in combination with Th1 and Th2 cytokines and chemokines orchestrate chronic allergic inflammation; the major cause for chronic morbidity of asthma