Basic Immunology

26th lecture:
Oral mucosal diseases
1. Autoimmune ulcerative diseases

2. Recurrent aphthous stomatitis

3. Oral candidiasis

4. Herpes Simplex infection
Oral epithelium

Built up of cells (mainly keratinocytes) + Basement membrane

Basement membrane: connects epithelium to lamina propria
Consists of: basal cell plasma membrane + lamina lucida + lamina densa + sublamina densa

Cell – cell connections: desmosomes + gap junctions, tight junctions

Cell – Basement membrane connection: hemidesmosome
Oral epithelium

Dsg 3 are expressed in skin but oral epithelium expresses predominantly Dsg 3 (Shirakata et al., 1998). This has consequences in terms of disease manifestations as discussed below as well as in antibody detection (Hashimoto, 2003).

The BMZ contains a mixture of structural components and antigens including type VII collagen, which is the major structural component of anchoring fibrils, and type IV collagen, which is a major component of vertebrate basement membranes, and shows diversity in its subunit composition. All the α chains contain three primary regions: an amino terminal 7S domain, a central triple helical portion interrupted in several areas by nonhelical segments, which provide the molecule with flexibility, and the collagenase-resistant carboxy-terminal globular domain (NC1) which contains 12 highly conserved cysteine residues.

Laminins are the most abundant noncollagenous glycoproteins of basement membranes and exist in a wide variety of molecular forms (Schéele et al., 2007). They are composed of genetically distinct α, β, and γ chains and 11 laminins are now recognized. The association of one α, one β, and one γ chain into large disulfide bonded heterotrimers determines the existence of the laminin isoforms, which have been renamed laminins 1 to 11 (Burgeson et al., 1994). Both circulating and bound antibodies against laminin 5 and more recently laminin 6 have been demonstrated in subsets of patients with mucous membrane (cicatricial) pemphigoid (Chan et al., 1997). These antibodies probably compromise interaction between the laminins 5/6 and the other basement membrane components and thus the integrity of this zone. Entactin (Nidogen) is a 150 kDa glycoprotein interacting with both laminin and type IV collagen. In addition there are a number of heparan sulfate proteoglycans, with molecular weights up to 400 or 500 kDa. The pemphigoid antigens are protein antigens. The main ones are 230 and 180 kDa, although several others have been described (see below).

BP1 is localized at the inner plate of the HD, while BP180 is a transmembrane molecule and part of the hemidesmosome-anchoring filament complex (Champliaud et al., 1996). An immunodominant antigenic site within the extracellular noncollagenous domain of this protein called NC16A is recognized by the majority of sera from pemphigoid patients. The α6β4 integrin is a further transmembrane constituent of the HD that binds with high affinity to laminin 5 while the α6 subunit is thought to interact with the NC16A domain of BP180 (Hopkinson et al., 1995). The epithelium thus has a complex structure (Figure 7) and an array of molecules is required for epithelial integrity and health (Table 3).

Historically, identification of the bullous pemphigoid antigen dates back to 1977 (Diaz et al., 1977). The existence of two major antigens, BP230 and BP180, has been confirmed by several independent groups; both appear to be components of the HD. The bullous pemphigoid antigen 1 (BPAG1/BP230) shares close sequence homology with the intermediate filament binding proteins plectin and desmoplakin I/II. Ultrastructurally BPAG1 is localized to the inner plate of the HD and does not appear to interact with the anchoring filament.
Epithelial and Basement membrane (auto)antigens

The skin shields the interior of a person from the external environment. It consists of three layers: epidermis, dermis, and subcutis. The epidermis is the outermost layer, responsible for physical protection and barrier function, while the dermis is the intermediate layer, providing structural support and containing blood vessels and nerves. The subcutis is the deepest layer, mainly composed of adipose tissue.

The skin's protective function relies on the stability of the cell connections within these layers. This stability is mediated by hemidesmosomes, which tie the cytoskeletons of the epidermal and dermal layers. Hemidesmosomes contain proteins such as Desmoglein 1/3, Desmocollin, and Periplakin.

Blisters can form within the epidermal layer (stratum spinosum) due to the disruption of these cell connections. The connection between the epidermis and dermis is facilitated by the basement membrane, which is composed of proteins such as laminin and type VII collagen.

Autoimmune disorders like pemphigus vulgaris, pemphigus foliaceus, and pemphigoid diseases are characterized by the formation of antibodies against these extracellular matrix proteins. These antibodies can lead to autoimmune skin reactions, resulting in blister formation and skin lesions.
Epithelial and Basement membrane (auto)antigens

Pemphigus vulgaris
Desmoglein 3 (important in desmosome)

Fig. 6
Epithelial and Basement membrane (auto)antigens

**Mucous membrane pemphigoid**
- Laminins: non-collagenous glycoproteins
  - laminin 5, laminin 6

**Bullous pemphigoid**
- BP180: transmembrane molecule
- BP230 (=BPAG1, Bullous pemphigoid antigen 1): hemidesmosome inner inner plate

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![Epidermal side](Epidermal_side.png)
![Dermal side](Dermal_side.png)

Fig. 5
Indirect immunofluorescence tests (IIFT) for dermatology

EUROIMMUN offers a wide range of different IIFT substrates as BIOCHIP/EUROPLUS mosaics for the differentiation of various autoimmune bullous dermatoses: tissues, transfected cells and EUROPLUS substrates.

A study from van Beek et al. (2012, Orphant J Rare Dis 7) on the Dermatology Mosaic 7 confirmed the high sensitivity and specificity of the substrates. 98.8% of 42 BP sera reacted with the basement membrane of the tissue substrates (oesophagus, salt-split skin), 100% with the EUROPLUS substrate BP180-NC16A-4X and 55% with BP230 (gC)-transfected cells. The Dsg1-transfected cells had a sensitivity of 90% for PF (n = 50), whereas 98.5% of PV sera (n = 65) reacted with Dsg3-transfected cells. The specificity of the substrates was between 98.2% and 100%.

Oesophagus, Dsg1

Oesophagus: detection of antibodies against prickle-cell desmosomes (pemphigus) and basal lamina (pemphigoid).

Salt-split skin, BP180

Salt-split skin: differentiation of autoantibodies against antigens of the epidermal (BP180, BP230) and dermal (collagen type VII, laminin 332, p200) sides of the skin.

Transfected cells, Dsg1

Transfected cells: Monospecific detection of antibodies against Dsg1, Dsg3 (pemphigus), BP230 gC (pemphigoid), and collagen type VII (EBA).
Recurrent aphthous stomatitis (RAS)

Characterized by oral ulcers
Heals spontaneously in 7-21 days
Prevalence: ~10%

Genetics:
~90% concordance in identical twins
Possible association with HLA-A2 and HLA-B12

Cause: ~unknown
(Definition: recurrent oral ulceration in the absence of known systemic factors…)

Hypothesis:
Unknown trigger (chemical or infective agent) → decrease in normal suppression → autoimmune response to oral mucosa
Recurrent aphthous stomatitis (RAS)

Findings:
- Autoantibodies against epithelial cells (leading to cell death)
- Cytotoxic T cells sensitized to oral mucosa

Trigger agent:
- Possibly cross-reacting with oral mucosa
- Candidate: heat-shock protein (HSP) 60kDa
- Microbial HSP → stimulate mucosal Langerhans cells → generation of T-cells that recognize microbial HSP + homologous human HSP

Several other types of (non-aphthous) oral ulcers with underlying causes

(Hematological diseases, gastrointestinal enteropathies, dermatological conditions etc…)

Differential diagnosis is important!
Oral candidiasis

Candida species: present in ~40% of population

Oral candidiasis: usually with underlying causes
   Immunosuppression: therapy, HIV
   Other oral diseases present
   Xerostomia

Main types:
   Acute pseudomembranous candidiasis (very young or elderly)
   Acute atrophic candidiasis (antibiotics)
   Chronic atrophic candidiasis (prosthesis)
   Chronic hyperplastic candidiasis (risk of malignant transformation)
   Erythematous candidiasis (HIV infection)
Mucosal immune response to Candida

Innate immune response: **polymorphonuclear** cells found in biopsies

Oral candidiasis present in 40% of HIV+, 75% of AIDS patients → role of **T cells**
- $T_H^1$: elevated IL-12, IFNγ observed in patients
- $T_H^{17}$: elevated IL-17 and IL-23 associated with protection
  - $T_H^{17}$-deficient patients are susceptible to oral candidiasis

IgA-deficiency: increased prevalence of oral candidiasis → role of **B cells**

  - Secreted aspartyl protease 2 (SAP2): important Candida antigen
    - Immunization against SAP2 → secretory IgA-type antibodies → protection in mouse model
Herpes simplex

Usually caused by Herpes simplex virus 1 (HSV1)

Prevalence: 58% between ages 14-49

Primary infection: *herpetic gingivostomatitis*
  - Children or young adults
  - Pathogenesis: lytic replication of the virus in epithelial cells → lysis of keratinocytes
  - Immune response: inflammation + adaptive (neutralizing antibodies + CD8+ T<sub>C</sub>)
  - Self-limiting in immunocompetent patients

Characteristic clinical appearance: ulceration of oral mucosa + malaise, fever

Therapy: acyclovir only at beginning of infection + symptomatic treatment
Herpes simplex

HSV1: Rapid transmission to peripheral sensory nerve fibers of n. trigeminus
Retrograde transport of the virus to trigeminal ganglion

Before appearance of neutralizing antibodies!!

Stays latent for years

Reactivation: in 15-40% of seropositive patients; appears as herpes simples labialis

Trigger factors: UV, stress, illness, immunocompromised conditions

Recurrence: usually in same spot
Herpes simplex labialis

Virus migration from neural cell body to periphery
  infects and replicates within keratinocytes
  keratinocyte death $\rightarrow$ inflammation $\rightarrow$ papule formation
  $\rightarrow$ vesicle formation

Resolve spontaneously in 7-10 days
  appearance of neutralizing antibodies
  $T_H$: produce IFN$\gamma$ and IL-12
  $T_C$: cytotoxicity (keratinocyte lysis!)