

Research & development projects

1. Psoriasis research

Our studies are aimed at investigating basic immunological mechanisms, e.g. cytokines and chemokines and their regulation through therapeutic interventions in inflammatory diseases like psoriasis in comparison to different forms of eczema. These investigations may help to identify new targets for future therapeutic intervention.

- Yawalkar N, Hunger RE, Buri C, Egli F, Brand CU, Mueller C, Pichler WJ, Braathen LR. A comparative study of the expression of cytotoxic proteins in allergic contact dermatitis and psoriasis: Spongiotic skin lesions in allergic contact dermatitis are highly infiltrated by T cells expressing perforin and granzyme B. *Am J Pathol* 2001; 158: 803-808
- Keller M, Spanou Z, Schaerli P, Britschgi M, Yawalkar N, Seitz M, Villiger PM, Pichler WJ. T cell-regulated neutrophilic inflammation in autoinflammatory diseases. *J Immunol* 2005;175: 7678-7686
- Hassan AS, Simon D, Simon HU, Braathen LR, Yawalkar N. Efalizumab-associated papular psoriasis. *Arch Dermatol* 2007;143:900-6

2. Translational medicine in the field of cell therapy (Chronic wounds and pigmentation)

Tissue engineering for skin wound treatment, i.e.

- autologous, ORS (= outer root sheath of plucked anagen scalp hair follicles)-derived keratinocyte transplantation [product Epidex]
- allogeneic, 2-cell-type (fibroblasts and keratinocytes) wound stimulation [product Allox]
- ORS-derived autologous melanocyte transplantation for skin depigmentation, e.g. vitiligo.

Prof. Dr. med. Thomas Hunziker is vice-chairman of the Department of Dermatology. One of his main fields of research is tissue engineering for the treatment of skin defects. He initiated and scientifically supervised the development, clinical testing and market introduction of two tissue engineering products, EpiDex and Allox. He is co-founder and past president of the Swiss Association for Wound Care.

- Tausche A-K, Skaria M, Böhlen L, Liebold K, Hafner J, Friedlein H, Meurer M, Goedkoop RJ, Wollina U, Salomon D, Hunziker T. An autologous epidermal equivalent tissue-engineered from follicular outer root sheath keratinocytes is as effective as split-thickness skin autograft in recalcitrant vascular leg ulcers. *Wound Rep Reg* 2003;11: 248-252
- Goedkoop RJ, Hunziker T. Allox, a growth-arrested, allogeneic cell-based treatment. For chronic venous leg ulcers. *WHS Seattle 2003. Wound Rep Reg* 2003;11:A19 Abstract 71
- Hunziker T: Review: Autologer kultivierter Hautersatz. *Hautarzt* 2004;55:1077-85

3. Cutaneous drug reactions

- The main research goals are an improved understanding of the molecular interaction of drugs/chemicals with immune cells, i.e. T cells, dendritic cells and how they stimulate (or inhibit) the immune system. These studies are planned to pave the way for improved methods to diagnose drug induced adverse reaction and to improve risk assessment of chemicals/drugs.
 - Yawalkar N, Hari Y, Frutig K, Egli F, Wendland T, Braathen LR, J. Pichler W. T cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug-specific and cytotoxic. *J Invest Dermatol* 2000; 115: 647-652
 - Yawalkar N, Shrikhande M, Hari Y, Nievergelt H, Braathen LR, J. Pichler W. Evidence for a role for IL-5 and eotaxin in activating and recruiting eosinophils in drug-induced cutaneous eruptions. *J Allergy Clin Immunol* 2000; 106: 1171-1176
 - Yawalkar N. Drug-induced exanthems. *Toxicology*. 2005; 209:131-134

4. Atopic eczema and contact dermatitis

Pathogenic mechanisms of chronic inflammatory skin diseases including eczema represent an important research area in our department. By analyzing the skin infiltrating cells and cytokines as well as their regulation, we aim to better understand the underlying pathophysiologic mechanisms of eczema. Within this research frame, the function of eosinophilic granulocytes is of particular interest. Investigating their pathogenic role in eosinophilic skin diseases will help to develop new therapeutic strategies.

- Simon D, Wittwer J, Kostylina G, Büttiker U, Simon HU, Yawalkar N. Alefacept (lymphocyte function-associated molecule 3/IgG fusion protein) treatment for atopic eczema. *J Allergy Clin Immunol*. 2008;122:423-4
- Simon D, Hösli S, Kostylina G, Yawalkar N, Simon HU. Anti-CD20 (rituximab) treatment improves atopic eczema. *J Allergy Clin Immunol*. 2008 ;121:122-8
- Hassan AS, Kaelin U, Braathen LR, Yawalkar N. Clinical and immunopathologic findings during treatment of recalcitrant atopic eczema with efalizumab. *J Am Acad Dermatol* 2007; 56:217-221

5. Acne inversa

Acne inversa (hidradenitis suppurativa) is a chronic inflammatory disorder of the apocrine gland-bearing skin. The clinical course can be devastating. End-stage acne inversa is disabling and has a profound impact on the quality of life. At present, the pathophysiology of acne inversa is still poorly understood. To better understand its mechanisms we are performing the following studies: 1) analysis of the expression of Toll like receptor 2 (TLR2) in lesional tissue, which seems to play an important role in maintaining the chronic inflammation; 1) characterization of the role of different subsets of macrophages (M1 and M2 subsets) and T cells (Th1 and Th2 cells) in the immune response. To this end, we have established a novel method to isolate the infiltrating cells from the lesions to perform flow cytometric analysis.

- Hunger RE, Surovy AM, Hassan AS, Braathen LR, Yawalkar N. Toll-like receptor 2 is highly expressed in lesions of acne inversa and colocalizes with C-type lectin receptor. *Br J Dermatol.* 2008 ;158:691-7
- Schlapbach C, Yawalkar N, Hunger RE: Antimicrobial Peptides in Acne Inversa: *Dermatologica Helvetica* 6/2008: P61 42 (abstract)
- Schlapbach C, Yawalkar N, Hunger RE. hBD-2 and Psoriasin are Overexpressed in Lesions of Acne Inversa (manuscript submitted)

6. Non-melanoma skin cancers

The research is focused on skin and oral squamous cell carcinomas. Our study aims to identify clinically suitable molecular markers with regards to metastatic properties, tumor development and tumor recurrence potential by combining proteomic (tissue microarray, immunohistochemistry, in situ Hybridisation, FISH) and genomic (cDNA array, RT-PCR) investigation methods. This approach should: 1) help to select high-risk patients who may benefit from more aggressive treatment and follow-up protocols and 2) might identify target genes for novel pharmacological intervention.

We are currently establishing cohorts of patients with NMSC in the local population:

- to evaluate the demographics and epidemiological data of the high-risk sub-population
- *to characterize genetic factors linked to a higher risk for developing skin tumors*, the advantage of Bern and the adjacent region being a strong genetic homogeneity
- to identify specific epigenetic factors: alimentation (the consumption,...), toxic exposure (arsenic,) that could be part of unknown risk or protection factors for NMSC in addition to sun exposure. In fact, some patients develop tumors in sun-protected areas.

- Albinger-Hegyí A, Hegyi I, Nagy I, Bodmer M, Schmid S, Bodmer D. Alteration of activator protein 1 DNA binding activity in gentamicin-induced hair cell degeneration. *Neuroscience.* 2006 ;137:971-80
- High frequency of t(14;18)-translocation breakpoints outside of major breakpoint and minor cluster regions in follicular lymphomas: improved polymerase chain reaction protocols for their detection. *Am J Pathol.* 2002;160:823-32
- Gerbig AW, Zala L, Hunziker T. Tumorlike eosinophilic granuloma of the skin. *Am J Dermatopathol.* 2000 ;22:75-8

7. Cutaneous T cell lymphoma

Primary cutaneous T-cell lymphoma (CTCL) represents a heterogeneous group of extranodal non-Hodgkin lymphomas of which mycosis fungoides (MF) and its closely related leukaemic variant, Sézary syndrome (SS), are the most common types. Transformed T cells in CTCL

are typically memory CD45RO+ CD4+ T cells, produce Th2 cytokines, and display skin homing receptors such as CLA (cutaneous lymphocyte antigen) and CCR4. These cells have increased skin homing potential, explaining in part the high affinity of these cells for the skin. We have previously analyzed the frequency and distribution of dendritic cells (DC) in lesions of CTCL. We are currently planning to vaccinate CTCL patients with a human telomerase specific peptide (hTERT). The enzyme telomerase is critically involved in tumor cell immortalization. Due to its relatively specific expression in a very broad range of tumor tissue, telomerase is an attractive target for tumor therapy. Our goal is to induce a T cell specific immune responses against the malignant cells and to characterize the immune response.

- Yawalkar N, Ferenczi K, Jones DA, Yamanaka K, Suh KY, Sadat S, Kupper TS. Profound loss of T-cell receptor repertoire complexity in cutaneous T-cell lymphoma. *Blood*. 2003;102:4059-66
- Hunger RE, Ochsenbein A, Kaelin U, Yawalkar N. Cutaneous T cell lymphoma: Dendritic cells are increased in dermal infiltrates and are in close contact with CD4+ tumor cells. *Dermatologica Helvetica* 6/2008: P3 24 (abstract)

8. Autoimmune blistering diseases: bullous pemphigoid and pemphigus

- Our group is implicated in studies aimed at understanding the *pathophysiologic mechanisms of pemphigoids and pemphigus*, a group of severe autoimmune blistering diseases of the skin and mucosae. These disease run a chronic course, are frequently difficult to treat and are associated with a significant morbidity and mortality. Overall, understanding the etiopathogenesis of pemphigus and pemphigoid may provide crucial additional insight into basic mechanisms leading from autoimmunity to autoimmune disease and may help to design more specific therapeutic strategies.

The **pemphigoids** include bullous pemphigoid (BP), gestational pemphigoid and cicatricial pemphigoid. They are a relatively common group of autoimmune blistering disorders associated with autoantibodies directed against two proteins of the cutaneous basement membrane zone, BP180 and BP230.

- The current project is aimed at: 1) characterizing the humoral and autoreactive T cell response to BP180 and BP230 in the disease course of the PEs; 2) identifying laboratory markers predicting disease activity and outcome; 3) developing diagnostic tools such as ELISA for the detection of patients' autoantibodies with high sensitivity and specificity.

Pemphigus is another severe autoimmune blistering disease of the skin and mucous membranes. There are two major types of pemphigus: pemphigus foliaceus (PF) and pemphigus vulgaris (PV). They are caused by the production of IgG autoantibodies directed

against cell-cell adhesion complexes, called desmosomes. Specifically, two transmembrane desmosomal proteins are characteristically targeted by patients' autoantibodies, desmoglein (Dsg) 1 and Dsg 3.¹

- Our current project represents a joined effort of seven European groups with the following goals: 1) to better define the immune pathogenesis of pemphigus utilizing two in vivo models of pemphigus with emphasis on autoaggressive T cells and their collaboration with autoantibody (autoAb) secreting B cells; 2) analysis of the autoAb-driven effector phase frequently involving "epitope spreading"; 3) characterization of the molecular events leading to intraepidermal blistering ; 4) analysis of the impact of therapeutic strategies such as the monoclonal antibody anti-CD20 (Rituximab) on the cellular and humoral autoimmune response in pemphigus and 5) definition and establishment of clinical parameters as valid measurements for the extent and activity of disease and which will be compared to serological markers and life quality assessment in pemphigus.

- Di Zenzo G, Grosso F, Terracina M, Mariotti F, De Pita O, Owaribe K, Mastrogiacomo A, Sera F, Borradori L, Zambruno G. Characterization of the anti-BP180 autoantibody reactivity profile and epitope mapping in bullous pemphigoid patients. *J Invest Dermatol.* 2004;122:103-10
- Thoma-Uszynski S, Uter W, Schwietzke S, Schuler G, Borradori L, Hertl M. Auto-reactive T and B cells from bullous pemphigoid (BP) patients recognize similar antigenic regions of BP180 and BP230. *J Immunol* 2006; 176:2015-23
- Hofmann SC, Thoma-Uszynski S, Hunziker T, Bernard P, Koebnick C, Stauber A, Schuler G, Borradori L, Hertl M. Severity and phenotype of bullous pemphigoid relate to autoantibody profile against the NH₂- and COOH-terminal regions of the BP180 ectodomain. *J Invest Dermatol* 2002; 119: 1065-73..
- Laffitte E, Skaria M, Jaunin F, Tamm K, Saurat JH, Favre B, Borradori L. Autoantibodies to the extracellular and intracellular domain of bullous pemphigoid 180, the putative key autoantigen in bullous pemphigoid, belong predominantly to the IgG1 and IgG4 subclasses. *Br J Dermatol* 2001; 144: 760-8.
- Laffitte E, Favre B, Fontao L, Riou S, Jaunin F, Tamm K, Saurat JH, Borradori L. Plectin, an unusual target antigen in bullous pemphigoid. *Br J Dermatol* 2001;144:136-8.
- Skaria M, Jaunin F, Hunziker T, Riou S, Schumann H, Bruckner-Tuderman L, Hertl M, Bernard P, Saurat JH, Favre B, Borradori L. IgG autoantibodies from bullous pemphigoid patients recognize multiple antigenic reactive sites located predominantly within the B and C subdomain of the COOH-terminus of BP230. *J Invest Dermatol* 2000; 114: 998-1004.

8. Characterization of the interactions between spectraplakins and IF proteins

The group is implicated in basic investigative studies aimed at understanding the *association of spectraplakins family members with various intermediate filament (IF) proteins* in various epithelia and striated muscle cells, its regulation and how IF-membrane attachments contribute to the organization of cytoarchitecture. We are focusing our attention on plectin (PL), desmoplakin (DP), and BPAG1-e/BP230, members of the spectraplakins family of cytolinkers. These proteins mediate the linkage of intermediate filaments (IFs) to other cytoskeletal systems and specialized membrane sites in a variety of cell types. These connections are critical for the maintenance of cell architecture and cell resilience.

Spectraplakins and IF proteins have recently been found to also have regulatory functions with profound effects on signaling pathways and the cell response to injury. Mutations in PL, DP, BPAG1 and IF protein genes cause a variety of devastating human diseases, attesting to the importance of these proteins. Our objectives are to assess: 1) how these spectraplakins and IF proteins interact with each other; 2) how posttranslational modifications such as phosphorylation regulate these interactions as well as 3) to identify the implicated kinases.

- Fontao L, Favre B, Riou S, Geerts D, Jaunin F, Saurat JH, Green K, Sonnenberg A, Borradori L. The interaction of the bullous pemphigoid antigen 1, desmoplakin, and plectin with intermediate filaments is mediated by distinct and specific sequences within their COOH-terminus. *Mol Biol Cell* 2003; 14: 1978-92
- Lapouge K, Fontao L, Champlaud MF, Jaunin F, Frias MA, Favre B, Paulin D, Green KJ, Borradori L. New insights into the molecular basis of desmoplakin-and desmin-related cardiomyopathies. *J Cell Sci* 2006; 119: 4974-85