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1 *Delayed Type Hypersensitivity mouse model* © Fraunhofer IME / Natasja de Bruin.

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DELAYED TYPE HYPERSENSITIVITY MOUSE MODEL

Dermatitis

Dermatitis is a broad term covering a variety of different inflammatory skin diseases. The etiology of widely prevalent atopic dermatitis (up to 15%) is unknown, but a genetically deficient skin epithelial barrier is a major factor. In allergic contact dermatitis (prevalence 7-10%), eliciting factors include local exposure of the skin to environmental agents such as natural rubber, metals (e.g. nickel) and plants, including poison ivy, as well as detergents, drugs, pollen or animal fur. These act as allergens or antigens, inducing immune responses which appear in the form of rashes, bumps and sometimes blisters when severe. Treatment includes allergen avoidance and topical glucocorticoids.

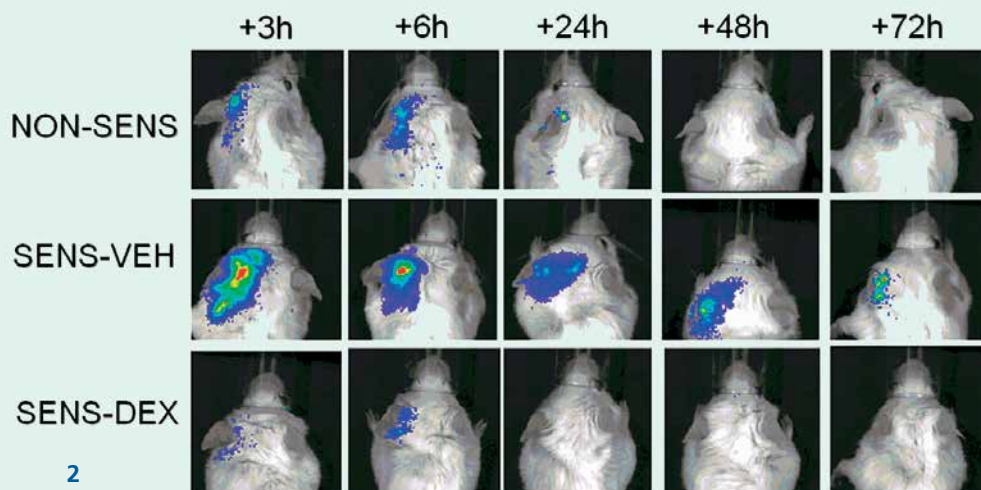
Animal models of dermal inflammation

Animal models of allergic contact derma-

titis and associated responses are useful for testing new therapeutic compounds, but also provide a simple means to study skin inflammation and systemic immune responses. For drug discovery and translational purposes, it is important that disease processes can be tracked in vivo over a relatively long period of time. In our opinion, in vivo bioluminescent imaging (BLI) is a valuable and reliable method for in vivo measurement of dermal inflammation and for the assessment of changes during resolution of inflammation. While mirroring to some extent changes in classical readouts such as histology and skin swelling, dynamic, time-dependent changes are only detectable with BLI.

Delayed Type Hypersensitivity

Allergic contact dermatitis is a T-cell-mediated hypersensitivity reaction (Delayed Type Hypersensitivity or DTH Type IV), an immune response which manifests an



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inflammatory reaction, due to activation of T cells and mononuclear phagocytes. It reaches peak intensity 24 to 48 h after the antigenic challenge. Memory T-cells are generated that persist for many months or years, sustaining the hypersensitivity to the antigen.

Endpoints/Outcome parameters

Within the Fraunhofer IME Branch for Translational Medicine and Pharmacology, **oxazolone** is applied to the abdomen (sensitization phase). One week later, the same substance is applied to one ear (challenge phase).

Ear thickness and luminol-based bioluminescent imaging of myeloperoxidase activity are the main outcome parameters.

The most important measures at 3h, 6h, 24h, 48h and 72h after the challenge with oxazolone include:

- Ear thickness with a digital micrometer
- Bioluminescent imaging: The IVIS Spectrum (Caliper Life Sciences) is used as optical imaging technology to facilitate non-invasive longitudinal monitoring of disease progression (e.g. inflammation), cell trafficking and gene expression patterns in living animals. Luminol-based Bioluminescence imaging (BLI), a measure of myeloperoxidase (MPO) activity is employed as an in vivo marker of inflammation.

The results from non-sensitized (NON-SENS) animals are compared with data from the sensitized vehicle-treated animals (SENS-VEH).

Histopathology and Fluorescence-Associated Cell Sorting analysis

- FACS / immunohistochemistry (IHC)-analysis of tissue and blood samples
- Analysis of profile of cytokines / chemokines / lipids in tissue and blood samples
- **Hematoxylin and eosin staining** of skin tissue sections
- Several inflammatory skin diseases are associated with enhanced vascularity and vascular hyperpermeability. The **vascular (hyper)permeability** (Evans blue) responses are investigated.
- **Multi-Epitope Ligand Cartography** allows multiple immunohistology by visualizing up to 40 antibodies on the same specimen. This is done in collaboration with the Institute of Clinical Pharmacology (Pharmazentrum Frankfurt/ZAFES, Frankfurt am Main).

Quality management and validation

The model has been validated with the clinical reference compound dexamethasone (DEX).

Selected publications

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- Homann J, Suo J, Schmidt M, de Bruin N, Scholich K, Geisslinger G, et al. In Vivo Availability of Pro-Resolving Lipid Mediators in Oxazolone Induced Dermal Inflammation in the Mouse. *PLoS One* 2015;10:e0143141. doi:10.1371/journal.pone.0143141.
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2 *Luminol-based Bioluminescence imaging, a measure of myeloperoxidase activity is employed as an in vivo marker of inflammation: effects of dexamethasone*
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