



CBI

Murine Atopic Dermatitis Model



COMPARATIVE BIOSCIENCES, INC.



Premier Preclinical Contract Research Organization

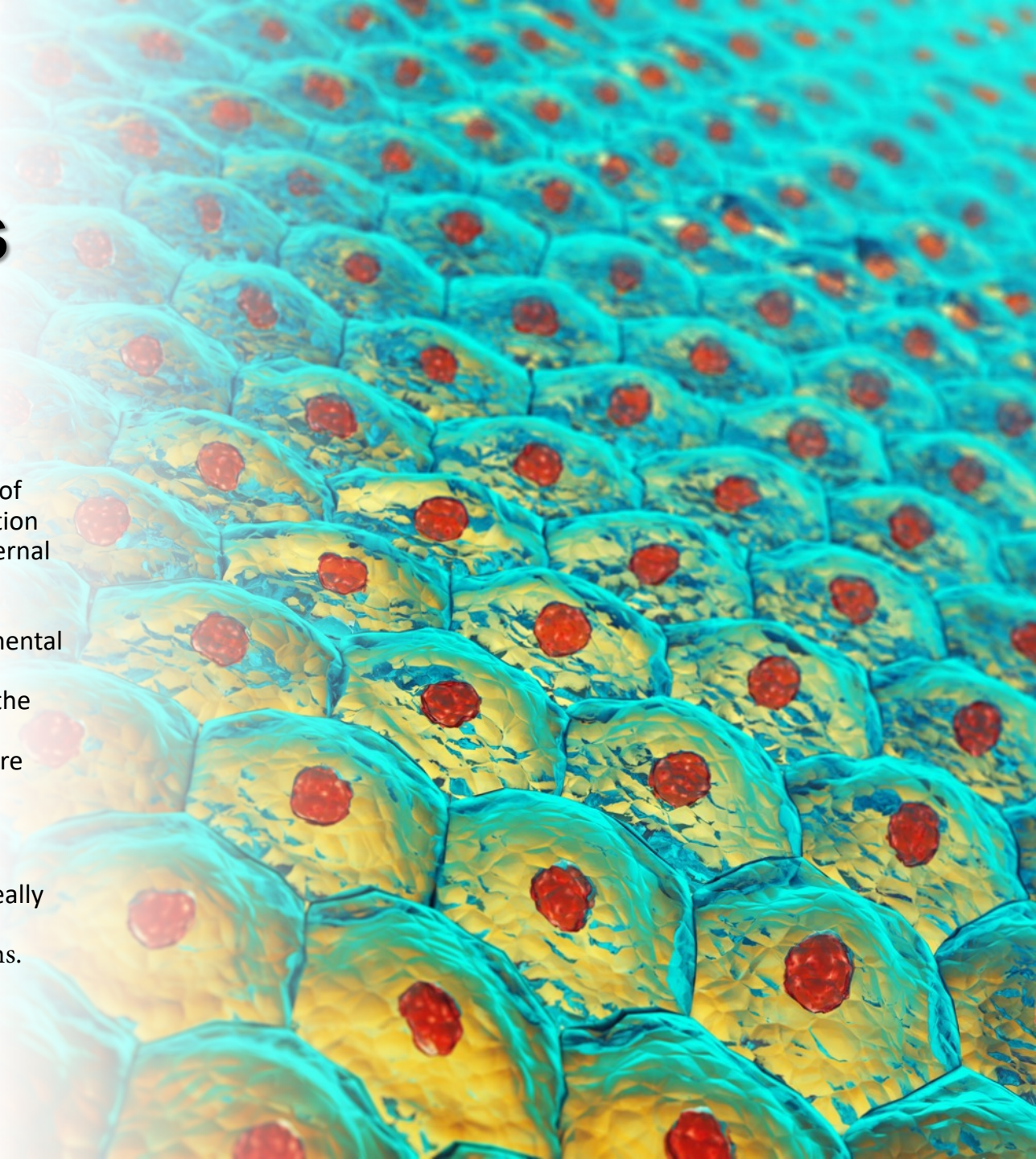
- Member of GD3 Genesis Drug Discover & Development Services
- **Over 25 years** of experience
- Conveniently **located in the heart of Silicon Valley**
- **State of the art, purpose-built facility**
- Approximately 35 employees
- Highly experienced scientific staff
- **GLP, OECD, FDA, USDA, OLAW**
- **AAALAC Accreditation**



Atopic Dermatitis Models

Atopic dermatitis has a complex etiology that involves dysfunction of the skin barrier, an abnormal reaction of the immune system against external antigens and/or autoantigens, and immunoglobulin (Ig)E-mediated sensitization to food and environmental allergens. A key hallmark of Atopic Dermatitis is disbalance between the Th1 and Th2 response, and animal models that mimic this response are more translational into the clinic.

Comparative Biosciences, Inc.'s combined dermatology and immunology expertise make us ideally suited to aid our clients Atopic Dermatitis drug discovery programs.



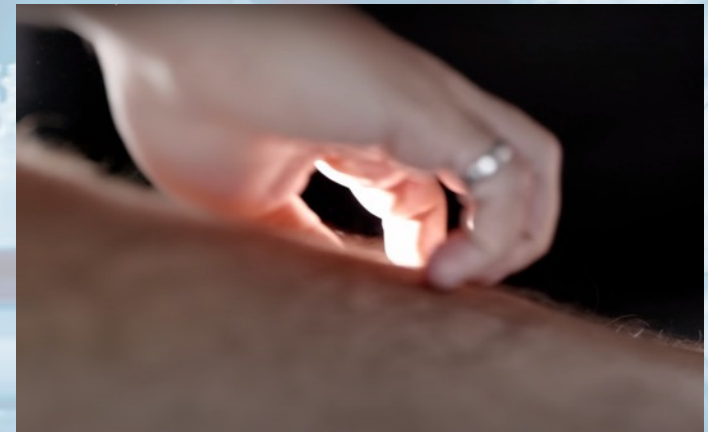


Atopic Dermatitis

Signs & Symptoms You
Must Not Ignore!

Atopic Dermatitis Models Overview

- Atopic Dermatitis (AD) is an inflammatory skin disease that frequently occurs in subjects with personal or family history of atopic disease.
- Mechanical injury to the skin by scratching is an important feature of AD and has been shown to induce local expression of IL-10 in patients.
- Epicutaneous sensitization with ovalbumin induction with tape stripping in mice is a useful model.
- This model displays many of the features of human AD, including scaly, inflamed skin, elevated total and specific IgE, histologic infiltration of CD3+ T cells and eosinophils in the dermis, and increased local expression of mRNA for Th2 cytokines.



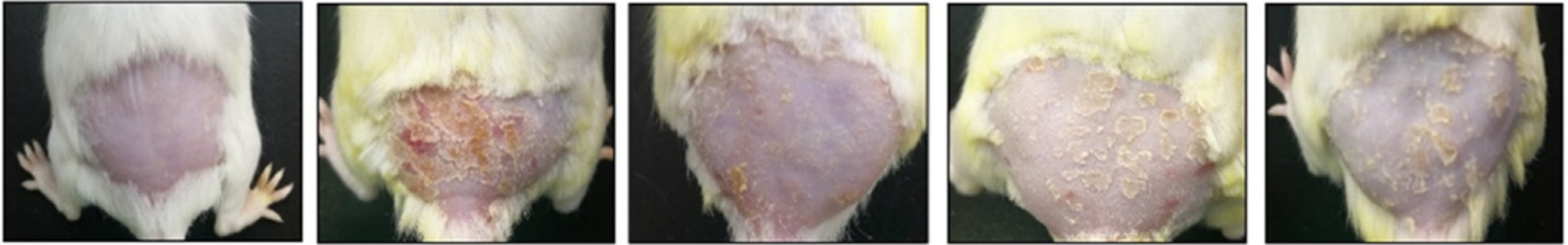
Basic Study Design

- Five groups of 10 mice per group; vehicle, test article at 3 dose levels, positive control.
- Housing (46 days on study plus acclimation) in dry environment
- Weekly body weights and daily clinical observations
- Induce atopic dermatitis by albumin and tape stripping at 3 intervals to back skin
Albumin is applied to tape-stripped skin for 1 week under a Tegaderm dressing followed by a 2 week rest period. Cycle is repeated 3 times
- Daily Draize scoring for up to 1 week during treatment phase to assess macroscopic changes.
- Treatment as per sponsor request – systemic or topical
- Necropsy and collect skin
- In-vitro assays as per sponsor request
- Histopathology of skin – special stains and assays as per sponsor request
- Complete report

Basic Study Design-Timing

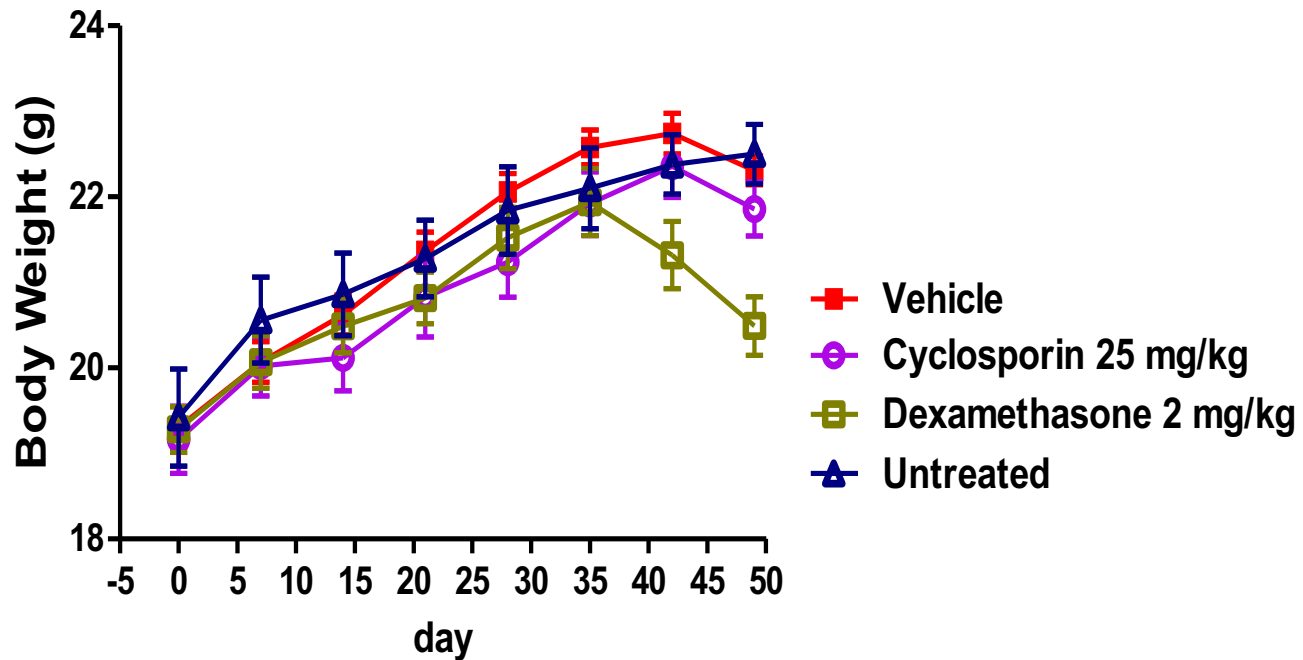
Tracking weeks	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Animals arrive [age to min 8 wks old]													
Sensitization													
Tape stripping (day 1)													
Apply ovalbumin (day 1-7)													
Rest period (2 weeks)													
Challenge													
Tape stripping (day 1)													
Apply ovalbumin (day 1-7)													
Dosing (day 1 - 7)													
Euthanize, Histopathology													



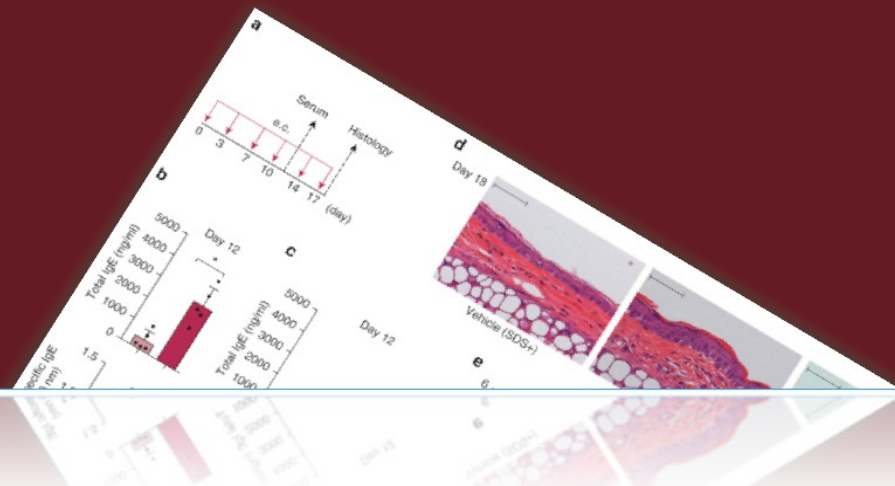


Body Weights

Body Weight (\pm SEM)

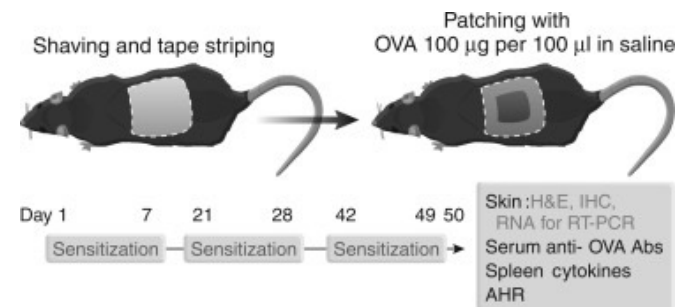


Histologic Effects



Ovalbumin sensitization and tape stripping produce

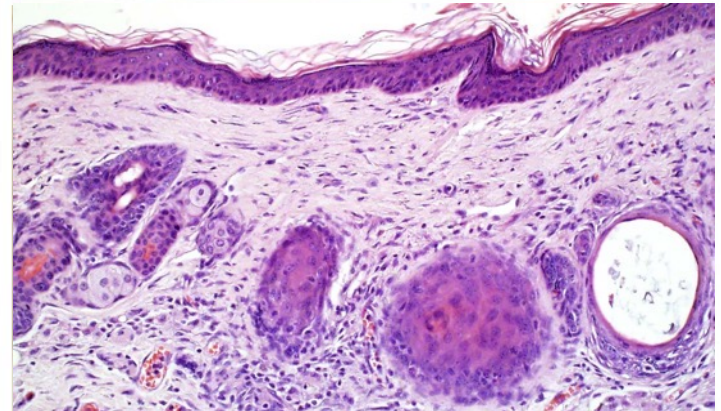
- **Macroscopic:** Variable scaliness, reddening, thickening, induration. In some cases, macroscopic changes are not particularly visible, although there are clear histopathologic changes
- **Histology:**
 - Acanthosis and hyperkeratosis of the epidermal layer.
 - Fibrosis and increased collagen in superficial dermis
 - Infiltration of dermis with eosinophils and mononuclear cells (Primarily T Cells). Chronic active multifocal to diffuse dermatitis in the tape stripped areas.
 - Inflammation may be located peri-vascularly or around the follicles
 - Edema and reactive changes in small vessels
 - Mast cells are increased in the areas of inflammation as are eosinophils



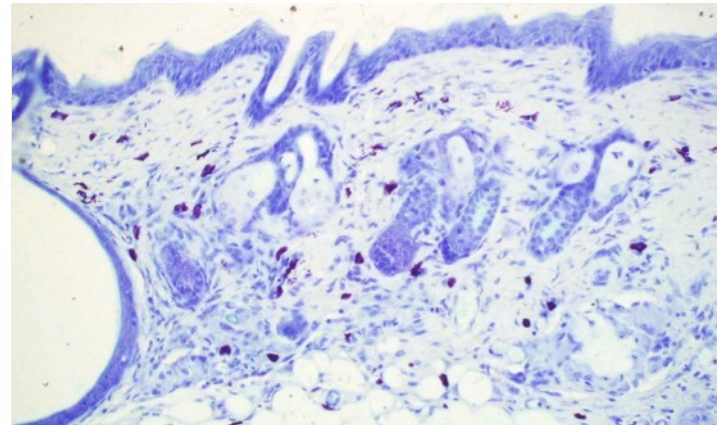
Histopathology in Mice



Appearance immediately post surgically



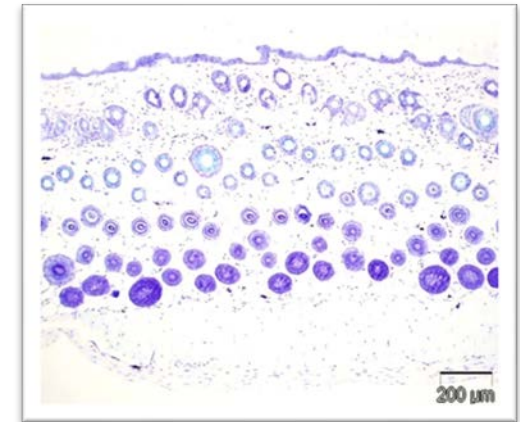
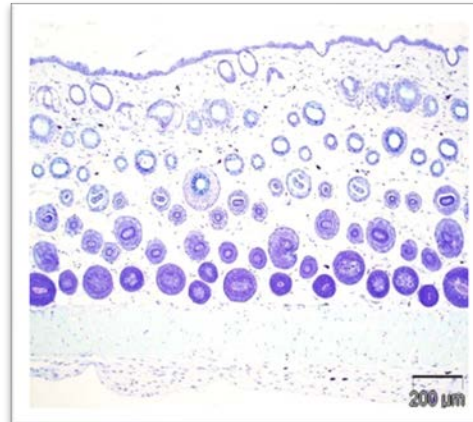
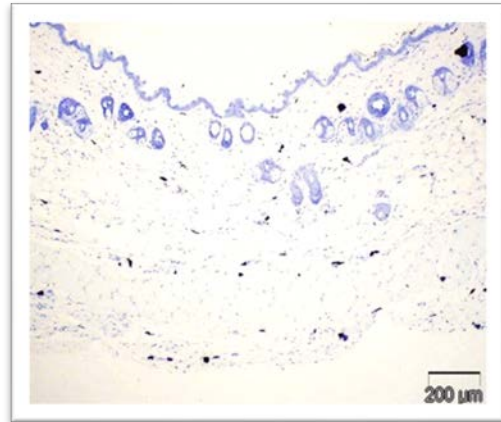
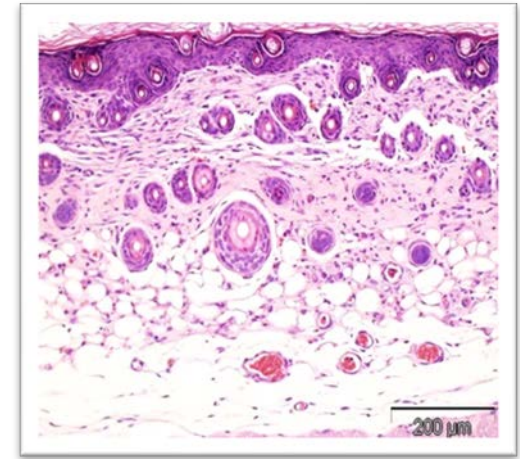
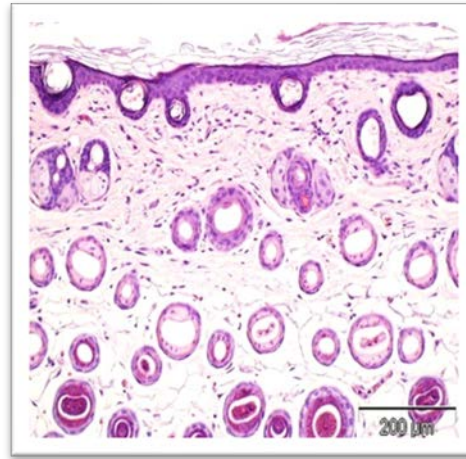
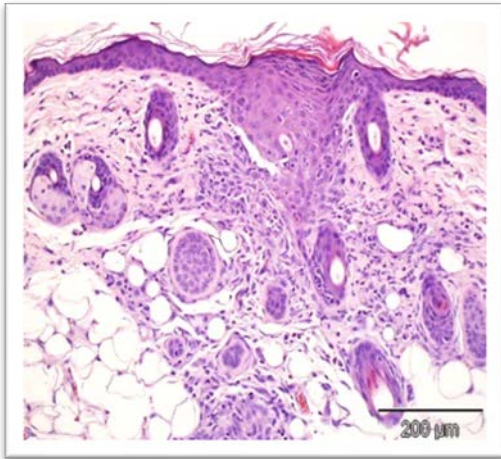
Typical dermal fibrosis, multifocal inflammation and epidermal hyperplasia found in this model.



The toluidine blue stain shows the increases in mast cells in the affected area.

The photomicrographs present the typical histopathology of this model in mice. The HE stain demonstrates the typical dermal fibrosis, multifocal inflammation and epidermal hyperplasia found in this model. The toluidine blue stain shows the increases in mast cells in the affected areas.

Histopathology



Vehicle

Cyclosporine

Dexamethasone

Cyclosporin and dexamethasone reduce inflammation and mast cells in atopic skin

Histology Inflammation Scoring



- 3-5 affected areas assessed histopathologically, H&E staining
- Scored semi-quantitatively:
0=normal, 1=minimal;
2=mild;
3=moderate;4=severe
- ^Statistically significant differences between groups

Histology Scoring	Tape Stripping & Saline Sensitization	Tape Stripping & Ovalbumin Sensitization	Subcutaneous Dexamethasone
	1	3	2
	2	3	2
	3	4	3
	2	4	2
	2	4	2
	1	3	2
	2	3	3
	2	3	3
	2	3	2
	3	2	1
Average	2.0	3.2[^]	2.2
SD	0.7	0.6	0.6

Dermal Mononuclear Cell Counts



- 3-5 affected areas assessed histopathologically, H&E staining
- Scored semi-quantitatively:
0=normal, 1=minimal;
2=mild;
3=moderate;4=severe
- ^Statistically significant differences between groups

Mononuclear cell counts	Tape Stripping and Saline Sensitization	Tape Stripping and Ovalbumin sensitization	Subcutaneous Dexamethasone
	56	96	66
	45	86	56
	48	70	76
	55	65	63
	41	69	62
	62	84	75
	64	90	57
	70	84	77
	41	97	64
	39	93	64
Average	52.1	^83.4	66.0
SD	10.9	11.6	7.6

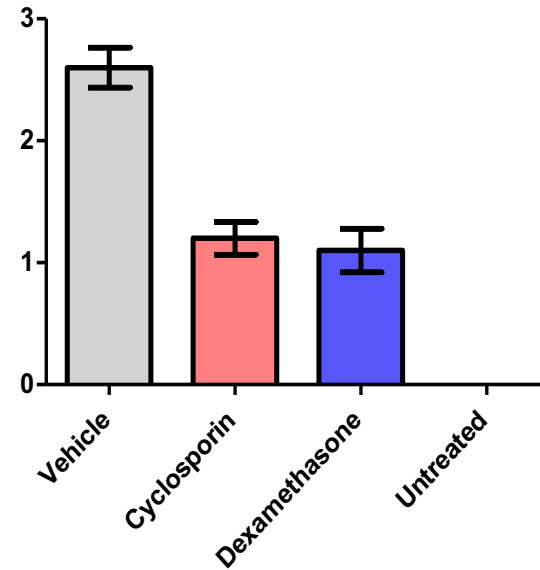
Data Graphs in Mice



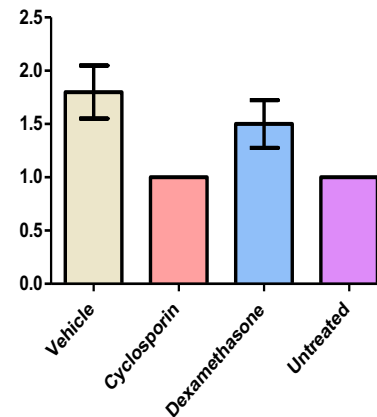
Mouse AD models can be categorized into three groups

1. inbred strains of mice that develop AD-like inflammation
2. general-engineered models with either ablation or overexpression of a single gene, either ubiquitously or in a certain cell lineage
3. AD-like inflammation induced by exogenous agents

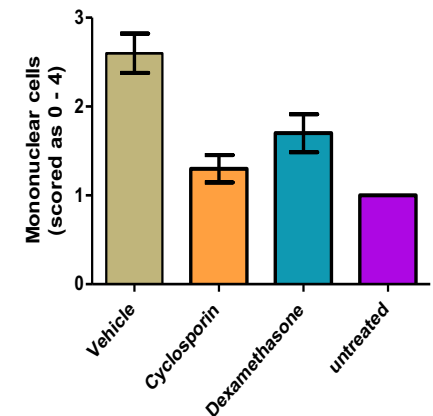
Inflammation (\pm SEM)



Mast cell degranulation (\pm SEM)



Monocyte infiltration (\pm SEM)



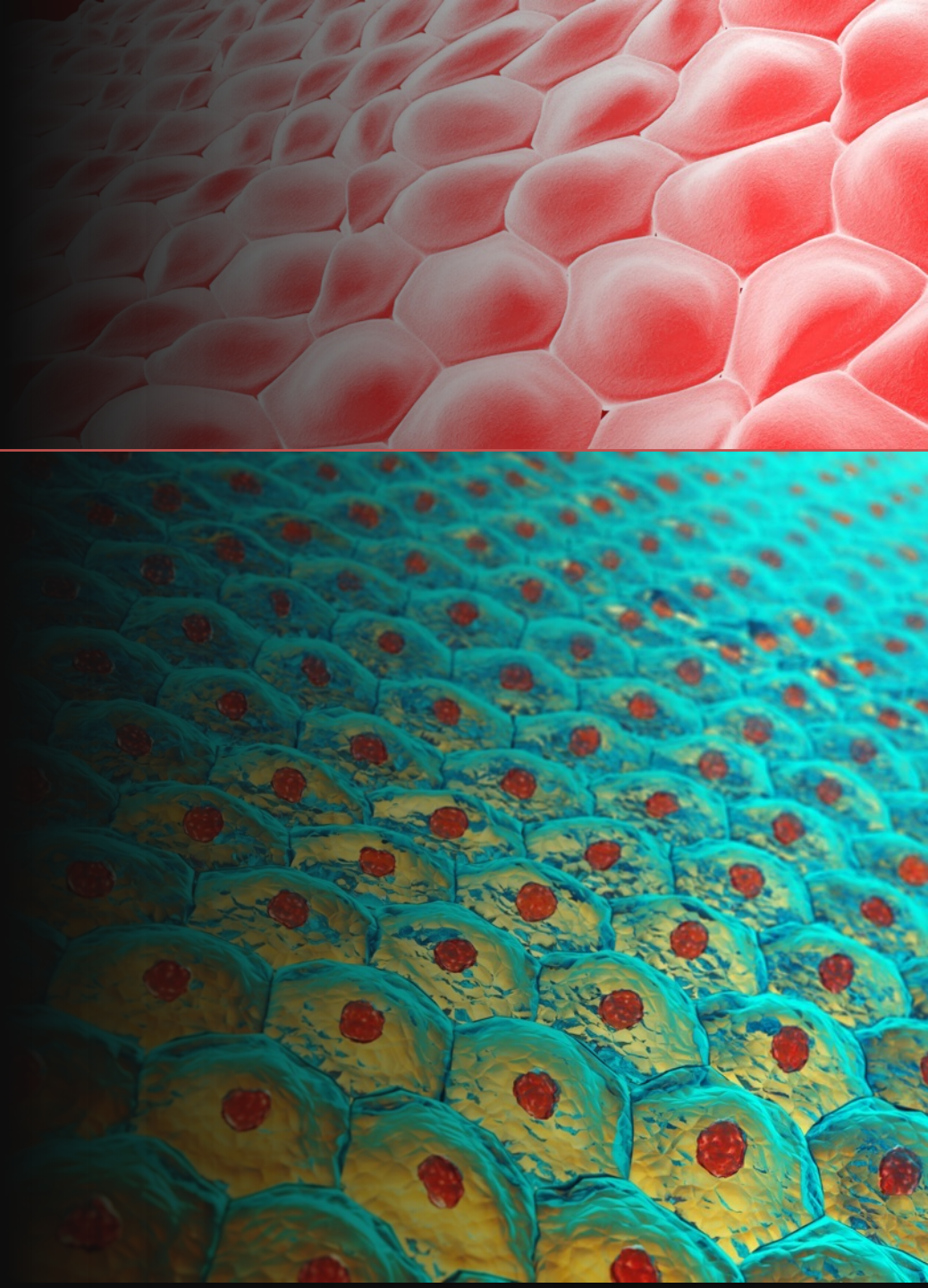
Summary

CBI provides a consistent, reproducible, validated tape stripping ovalbumin sensitization model in mice as a model for atopic dermatitis

Characterized by variable dermal thickening, scaliness and reddening macroscopically

Dermal chronic inflammation with epidermal thickening, dermal fibrosis, increased eosinophils, mast cells and mononuclear cells, and edema histopathologically

Dexamethasone provides amelioration of signs and histologic lesions.



Service and Quality

- ***Thoroughness in planning and execution is key to a successful study.*** All protocols are vetted and approved by multiple personnel. Our QAU has a rigorous training program. All non-GLP studies are conducted in the spirit of GLP.
- ***We believe in sound science.*** Our ratio of scientists to non-scientists is one of the highest in the industry. Every study director is a PhD-level scientist.
- ***We believe in communication.*** Timely responses to your inquiries and frequent updates on your study are mandatory.
- ***We welcome visitors.*** You are always welcome at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.

