Severe Skin Diseases: 
Integrating new concepts of basic research into a clinical perspective

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• Introduction

• Dendritic cells, Autophagy and Pathogens

• Skin cancer: Melanoma and Cutaneous lymphoma

• Clinical perspectives
Several pathological processes lead to severe skin diseases

Severe skin diseases and severe diseases with an important involvement of skin and mucosal tissues are frequent when combined
Cell types in the skin are at the core of the pathological processes.

- Epidermis
- Dermis
- Hypodermis
- Keratinocytes
- Melanocytes
- Dendritic Cells

O. Schwartz, Institut Pasteur, Paris

Dendritic cell (in blue) interacting with a lymphocyte (in pink)
A major role for the skin is protection

1. Mechanical

2. Thermal, chemical

3. Liquid loss

4. Pathogens (bacteria, viruses…)

5. UV radiation

- Mechanical
- Thermal, chemical
- Liquid loss
- Pathogens (bacteria, viruses…)
- UV radiation

- Healthy skin
- Skin with barrier alterations (atopic eczema)
- Skin with defects in DNA repair (XP)

Inflammation, infections

XP: Tumors
Dermatology has become a cross-disciplinary field

Historically based mainly on morphology

Towards a medical and surgical field integrating patient-oriented clinical and basic research
Dermatological science is rapidly evolving

19th
Visual diagnosis
Historical “treatments”
(Alibert, 1832)

20th
Clinical/dermatopathology
Non-targeted therapies
(corticosteroids)

21st
Mechanisms of diseases and
Targeted therapies
Targeted therapies,
biologics

(Alibert, 1832)
Hebra, 1856
An integrated approach is required for complex skin diseases

- Targeted therapies for the patient
- Clinical observation
- New therapeutic strategies
- Dermatopathology
- Clinical research
- Basic/translational research
Outline

• Introduction

• Dendritic cells, Autophagy and Pathogens

• Skin cancer: Melanoma and Cutaneous lymphoma

• Conclusions and perspectives
Dealing with skin complications of HIV infection

Great frequency in VIH+ patients and complex cases

Intermediate Immunosuppression

- Tuberculosis
- Herpes Zoster, Herpes
- Lymphoma
- Kaposi

Severe Immunosuppression

- PCP
- Toxo
- CMV
- MAC
Dendritic cells are targets of pathogens during mucosal transmission

Figure 2. Microbicides against HIV-1/herpes simplex virus type 2 (HSV-2)/human papillomavirus (HPV). Microbicides for HIV-1 (left), HSV-2 (center), and HPV (right). Microbicides inhibiting STI transmission are active either on or directly beneath the mucosal surface. Nonspecific microbicides, such as detergents, usually act in the first steps of viral contact with the mucosal barrier. Specific compounds generally impair viral binding or entry into host cells. Finally, highly specific microbicides act at later stages, such as non-nucleoside reverse transcriptase inhibitor-based microbicides against HIV-1. Alternative methods, such as RNA-interference-based microbicides against HSV-2, are also depicted.

Reviewed in Nikolic and Piguet, J Invest Dermatol, 2009
Dendritic Cells: sentinels of the immune system

- Defense against pathogens
  - Viral infections (HIV, HPV, HSV)
  - Bacterial infections (S.Aureus)
  - Yeast infections (candida)

- Immune response against tumors
  - Lymphomas
  - Melanoma (DC- based vaccines)

- Skin Diseases
  - Eczema
  - Atopic Dermatitis
  - Psoriasis
  - Lupus Erythematosus

Mechanisms of pathogens mucosal transmission

HIV: a paradigm for mucosal transmission

• Pathogens interact with mucosal tissues and other cellular targets in order to invade the host

• Study of transmission of HIV and other STI

Piguet et al., Cell, 1999
Piguet et al., Nature Cell Biol, 2000
Piguet and Sattentau, J Clin Invest, 2004
Piguet and Steinman, Trends in Immunology, 2007
Virological studies: tracking HIV in Dendritic Cells

Dapi  eGFP vpr  S15 mCherry

De Witte et al, Nature Medicine, 2007
Pion et al, J Invest Dermatol, 2007
Garcia, Traffic, 2008
Mangeat, PLoS Pathog, 2009
Live confocal studies: following viral particles across infectious synapses

Speed of HIV-1 Transfer:
0.40 +/- 0.23 μm/s
How does HIV escape full degradation in Dendritic Cells?

Are Lysosomes, autophagosomes, amphisomes involved?

Does HIV modulate autophagy in Dendritic cells?

What are the consequences for HIV antigen presentation?
Autophagy or cellular self-digestion is a cellular pathway involved in protein and organelle degradation

Myopathies
- **Pro**: Autophagy prevents aggregate-prone protein accumulation that leads to physiological dysfunction.
- **Con**: Autophagy may contribute to muscle wasting and defective autophagosome clearance may interfere with cellular function.

Ageing
- **Pro**: Autophagy removes damaged organelles and can limit production of reactive oxygen species.

Liver disease
- **Pro**: Autophagy can alleviate endoplasmic reticulum stress by degrading portions of the organelle containing misfolded proteins.
- **Con**: Excessive autophagy may cause liver damage.

Heart disease
- **Pro**: Autophagy may be protective during ischaemia and pressure overload.
- **Con**: Autophagy is harmful during reperfusion.

Cancer
- **Pro**: Autophagy acts in tumour suppression by removing damaged organelles and possibly growth factors, and reduces chromosome instability.
- **Con**: Autophagy acts as a cytoprotective mechanism that helps cancer cells resist anti-cancer treatments and survive in conditions of low nutrient supply.

Neurodegeneration
- **Pro**: Basal autophagy is a homeostatic process that prevents intracellular proteins from accumulating to toxic levels.
- **Con**: Insufficient lysosomal clearance results in intracellular accumulation of autophagosomes, which may process the amyloid precursor protein into toxic forms.

Infection and immunity
- **Pro**: Intracellular bacteria, viruses and proteozoon are removed from host removed from host cells by autophagy, and antigens are processed for MHC class II presentation. Autophagy may prevent auto-immune and inflammatory diseases.
- **Con**: Some microbe have have evolved to subvert autophagy to establish a replicative niche.

**Autophagy induction**
- starvation
- growth factor deprivation
- immune signals
- IFN-γ
- TNF
- TLRs
- PKR-eIF2α kinase
- Jnk
- FADD
- immunity-related GTPases

**Autophagy suppression**
- nutrient abundance
- insulin-Akt-TOR signaling
- immune signals
- IL-4
- IL-13
- FADD
- immunity-related GTPases


Autophagy is involved in several immunological processes

HIV-1 accumulates in a CD81-rich viral compartment (in part accessible from the cell surface)

Garcia, 2005, Traffic
Wilflingseder, 2007, J Immunol
Garcia, 2008, Traffic
Dendritic cells transfer HIV infection to CD4+ T cells across infectious synapses

Garcia, Traffic, 2008
Arhel, J Clin Invest, 2009
Profound loss of autophagosomal LC3-II in HIV-infected DC
Env mediated signaling in DC leads to activation of mTOR and Autophagy Block

phospho-S6 correlates with 1) mTOR activation and 2) a block in autophagy initiation

mTOR target: Phospho-S6

Adapted From Mizushima, Nature 2008

IB : anti-phospho proteins

IB : anti-actin

Phospho-Erk

MW

MW

MW

MW

MW

MW
HIV is routed to lysosomes via autophagosomes in DC.
Autophagy inhibition Increases DC-associated Virus

C

D

anti-GAG
LC-3 depletion in DC enhances viral transfer to T cells
DC activation via TLR is required for efficient antigen presentation: any role for autophagy?

Autophagy may be involved in the activation of innate immunity by delivering viral nucleic acids to endosomal compartments containing Toll-like receptor 7 (TLR7), which signals the induction of type 1 interferon (IFN) production.

From Levine and Kroemer, Cell, 2008
- HIV is routed via a novel specialized endocytic structure in DC: “immunoamphisosomes” (amphisomes = fusion between autophagosomes and endosomes)

- We propose that “Immunoamphisosomes” in DC: 1) amplify virus degradation and 2) enhance innate and 3) adaptive immune responses

- Restoring autophagy (via mTOR inhibitors) in DC increases HIV degradation and HIV antigen presentation on MHC-II

- Autophagy has implications for early events of HIV infection and rational vaccine design
Results from our studies: New lead candidates for intervention

- Langerin upregulation
- DC-SIGN / Cdc42 inhibition (Secramin A, Rho gtpases inhibitors)
- Autophagy (mTor inhibitors, sirolimus, everolimus...)
- APOBEC3G/F upregulation
Enhancing vaccine potential by encapsulating antigens and autophagy inducers (mTor inhibitors) into nanoparticles for transdermal delivery.

Figure 1. Scanning electron microscopy image of a single microneedle, optical microscopy image of the microneedle array, and schematic presentation of the application process.

Adapted from Alexander K. Andrianov, Apogee, Boston.
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Melanoma is
- the most frequent tumor in women aged 25-29
- the third most frequent tumor among 20-39 (M+F)

Tumor type that leads to high numbers of years lost, only behind CNS tumors
Nevus

Atypical Nevus

Malignant Melanoma

Metastatic disease
Translational research: Global genomic Analysis of melanoma

Genetic signatures
- Mechanisms of tumor formation
- Prognostic markers
- Therapeutic targets

Atypical Nevus

Excision

Extraction RNA

Alternative Splicing DATAS Gene Expression microarrays

Collaborations:
- Stecca, PNAS, 2007
- Preynat-Seauve, Cancer Research, 2007
Validation of gene candidates potentially involved in tumor progression: BCSC-1

Loss of expression of BCSC-1 in metastatic melanoma
Better survival in patients correlates positively with BCSC-1 expression.
BCSC-1 reduces melanoma cells proliferation (block in G2/M) but increases their migration.
BCSC-1 modulates ERK signaling and MITF

A. [Diagram showing gene expression data]

B. [Heatmap indicating high and low expression levels]

C. [Venn diagram with categories of cell proliferation, cell cycle progression, migration & adhesion, and tumor progression]
Therapeutic applications and melanoma genomics: Targeted therapies for melanoma

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• Conclusions and perspectives
• Heterogeneous group of B and T neoplasia affecting primarily the skin

• Low grade lymphomas but aggressive forms (f.i. Sezary)

• Survival depends on early diagnosis

• Mechanisms still unknown: tumor-host interactions
Clinical features, Histology and immunohistology: major criteria

Molecular biology (T/B cell receptor rearrangements Skin/blood)

Immunology: flow cytometry – Abnormal lymphocytic populations
• Analysis of function of the immune system polarization during CTCL: Th2

• Sezary syndrome: deficit in CD40 ligand
  (collaborations: Huard and al, Blood, 2005)

• Study of a subgroup of mycosis fungoides associated with neutrophilic reactions

Adapted from Immunity, 2008
CTCL with neutrophilic reactions: IL-17

• patients Geneva-Paris (French CTCL group): subgroup of CTCL with poor prognosis

• Modulation of Th-17 cytokines

Fontao et al., in preparation
**Already in the clinic:**
- Alpha-Interferon
- Rexinoids (bexaroten)
- Photopheresis
- monoclonals (anti-CD20, CD52, CD4, CD25…)

**In development:**
- HDAC inhibitors (modulation cytokines Th2)
- mTor inhibitors (everolimus)
- New monoclonals

**Therapeutic implications:** Immunomodulation during B or T cell cutaneous lymphomas

**Monoclonal antibody**

**Cutaneous B cell Lymphoma**

**Complete remission**
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Conclusions

1. Investigative Dermatology is a broad scientific discipline including but also extending beyond “classical” skin diseases (eczema, psoriasis…)

2. Several of the basic mechanisms we have studied have implications that apply to other skin conditions (f.i mTOR inhibitors) as well as other areas of medicine (infections/immunology)

3. Projects involving translational aspects (clinical-research) require a close collaboration between the laboratory and the clinicians
Future areas of investigation in Clinical and Investigative Dermatology

Basic Research

- Dendritic Cells and pathogens – HIV, herpes

Translational Research

- Nanoparticles, intradermal vaccination
- Syphilis: qPCR, Epidemiology

Clinical Research

- Melanoma: genomic approaches
- Cutaneous lymphoma: immunology
- Clinical studies, case reports
Local and international collaborations to support a dynamic program

Collaborations
Clinical studies, areas of excellence

Cross-functional Collaborations
Infectious diseases, oncology, immunology, hematology
- Pediatric
- Obgyn
- Geriatric
- Surgery
- Laboratories, Pathology

Research
Basic, Translational, Clinical

University
Hospital
Dermatology Department

National, EU, outside EU

Medical societies:
National, ESDR, EADV…

Funding opportunities:
(govt/EU); Foundations

Pharma, Biotech
Main events for investigative dermatology and sister societies

2010  40th Annual ESDR Meeting
Helsinki, Finland
8-11 September 2010

2011  41st Annual ESDR Meeting
Barcelona, Spain
7-10 September 2011

2012  42nd Annual ESDR Meeting
Venice, Italy
19-22 September 2012

2013  International Investigative Dermatology
Edinburgh, Scotland
8-11 May 2013
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