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d'actualisation
en dermato-allergologie

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Lyon-Sud →



IMMUNOLOGY OF SKIN ALLERGY / SKIN VACCINATION

Research activities

Pathophysiological research

Skin allergic diseases



Allergic contact dermatitis (ACD)



Atopic dermatitis (AD)

ECZEMAS



MILD - Exanthema



SEVERE - Blistering disease

DRUG ALLERGY

Translational research

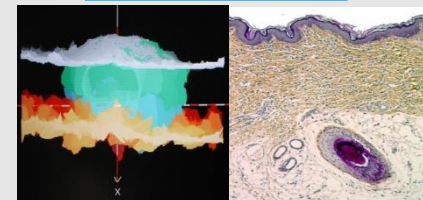
New immunological assays

Diagnostic



Prediction of allergenicity

Intradermal vaccination



Fragrance allergy

We know very little!

1. Fragrances = allergens?
2. Some fragrances = allergens of concern?
3. Is it useful to ban allergens of concern?
4. Is it necessary to reduce the prevalence of ACD to fragrances?
5. Is it necessary to reduce the prevalence of fragrance contact allergy?
6. Fragrance contact allergy = positive patch tests could be irritation or allergy
7. Contact dermatitis to fragrance = eczema could be irritation or allergy

Lack of evidence for allergenic properties of coumarin in a fragrance allergy mouse model

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ARIELLE GARD³ AND JEAN-FRANÇOIS NICOLAS^{1,2,4}

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Background: There is controversy as to whether coumarin, an ingredient in cosmetics and fragrances, is a contact allergen involved in fragrance allergy. We recently showed that the purity of coumarin is a critical parameter for its allergenicity because coumarin preparations containing trace amounts of contaminants induced cell proliferation in the local lymph node (LN) assay whereas pure coumarin did not.

Objective/Method: In the present study, we analyzed the sensitizing properties of coumarin (purity > 99.9) and of dihydrocoumarin (DHC), in a recently developed model of fragrance allergy in mice.

Results: DHC was able to prime T cells in LNs draining the sensitization skin site and to induce a typical allergic contact dermatitis (ACD) reaction upon challenge, confirming that DHC is endowed with moderate sensitizing properties. In contrast, no T-cell activation and no ACD responses were obtained following sensitization and challenge with coumarin.

Conclusion: These results confirm that pure coumarin is endowed with very weak sensitizing capacities, if any, and suggest that the presence of contaminants in coumarin preparations may account for the previously reported allergenic properties of coumarin.

Key words: allergic contact dermatitis; coumarin; fragrance allergy; mouse ear-swelling test; murine model; T cell priming. © Blackwell Munksgaard, 2007.

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The Skin Allergenic Properties of Chemicals May Depend on Contaminants – Evidence from Studies on Coumarin

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Table 3. Clinical studies of the allergenicity of pure coumarin

Studies	Centers	Length of the study months	Patients	Contact dermatitis	ACD FM+ ¹	ACD perfume+ ²	ACD atopy+ ³	Coumarin+/ total ⁴
Study 1 contact dermatitis	1	3	279 100	279 100	ND 3	ND 2	ND 12	0/279 0/100
Study 2 FM allergic	20	14	101		101	ND	22	1/101
Study 3 perfume allergic	7	12	30			30	5	0/30

ND = Not determined.

¹ Clinically relevant positive patch test to FM.

² Patients with a personal history of contact allergy to fragrances.

³ Patients with a personal history of atopic diseases.

⁴ Number of patients with a positive patch test to coumarin in the tested population.

Table 4. Frequency of positive patch test reactions to FM and coumarin over recent years in published studies on dermatological patients

Authors	Year	Patients	Positive patch test reactions, %
<i>FM</i>			
Uter et al. [27]	1992	6,700	7.4
	1996	9,600	10.3
Johansen et al. [28]	1985–1986	1,232	4.1
	1997–1998	1,267	9.9
Marks et al. [29]	1992–1994	3,509	11.4
	1994–1996	3,082	14.3
<i>Coumarin</i>			
Malten et al. [13]	1984	182	6.8
Kunkeler et al. [10]	1998	1,400	4.1
Frosch et al. [14]	2002	1,854	0.3
This study	2005	510	0.2

ALLERGIC CONTACT DERMATITIS

Contact Hypersensitivity



Repeated contact with skin sensitizers called haptens

HAPTENS

Non protein chemicals
Interact with aminoacid residues

DNP et TNP: lysin
Ni: histidin

- **Strong H**: DNP, TNP, oxazolone
ACD in 90% of people

- **Weak H** : metals (Ni, Cr, Cu)
ACD in 20% of people

- **Very weak H**: ACD in < 1%
Professional ACD, drugs



Contact dermatitis/ eczema
= skin inflammation

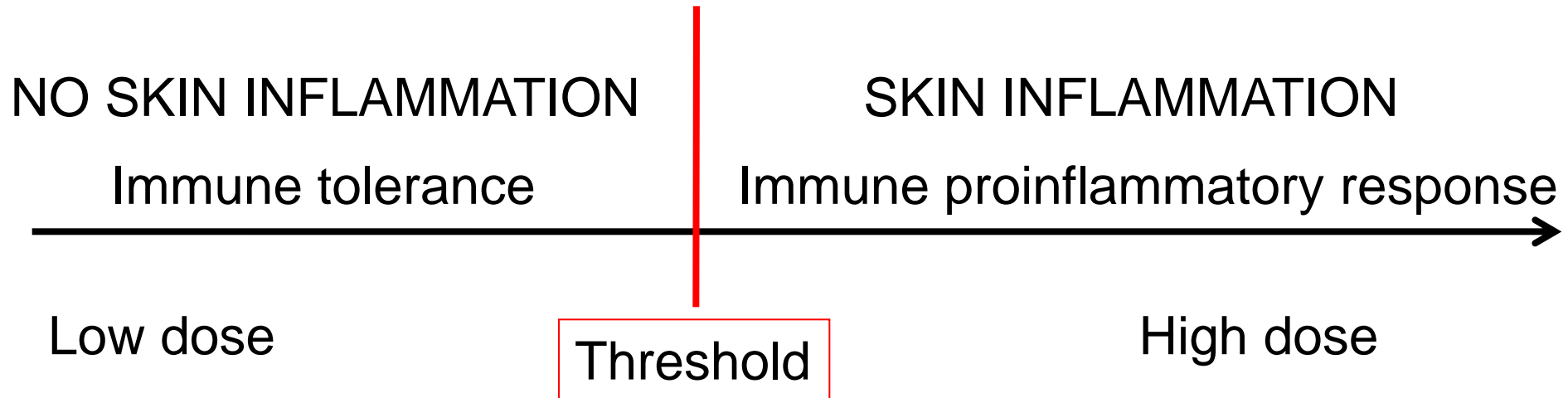
- | | |
|--|---|
| <ul style="list-style-type: none"> • Allergic CD • Specific T cells • CD8+ T cells/IFNγ • Sensitization & disease | <ul style="list-style-type: none"> • Irritant CD • No specific T cells • No CD8+ T cells/IFNγ • No sensitization |
|--|---|



Pos patch test = Skin inflammation

- | | |
|--|---|
| <ul style="list-style-type: none"> • True + • Contact allergy = allergy to patch test • Specific T cells • CD8+ T cells/IFNγ • Sensitization | <ul style="list-style-type: none"> • False + • Contact irritation • No specific T cells • No CD8+ T cells/IFNγ • No sensitization |
|--|---|

Take home messages



Scoring (ICDRG)



Négative



IR irritative



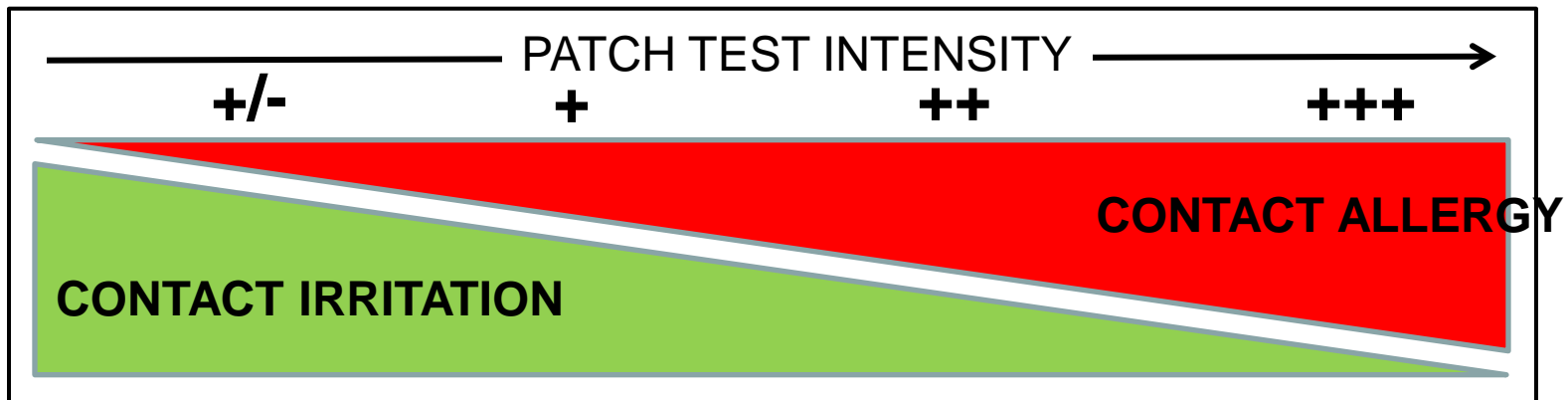
Positive +



Positive ++



Positive +++



Fragrance Allergy

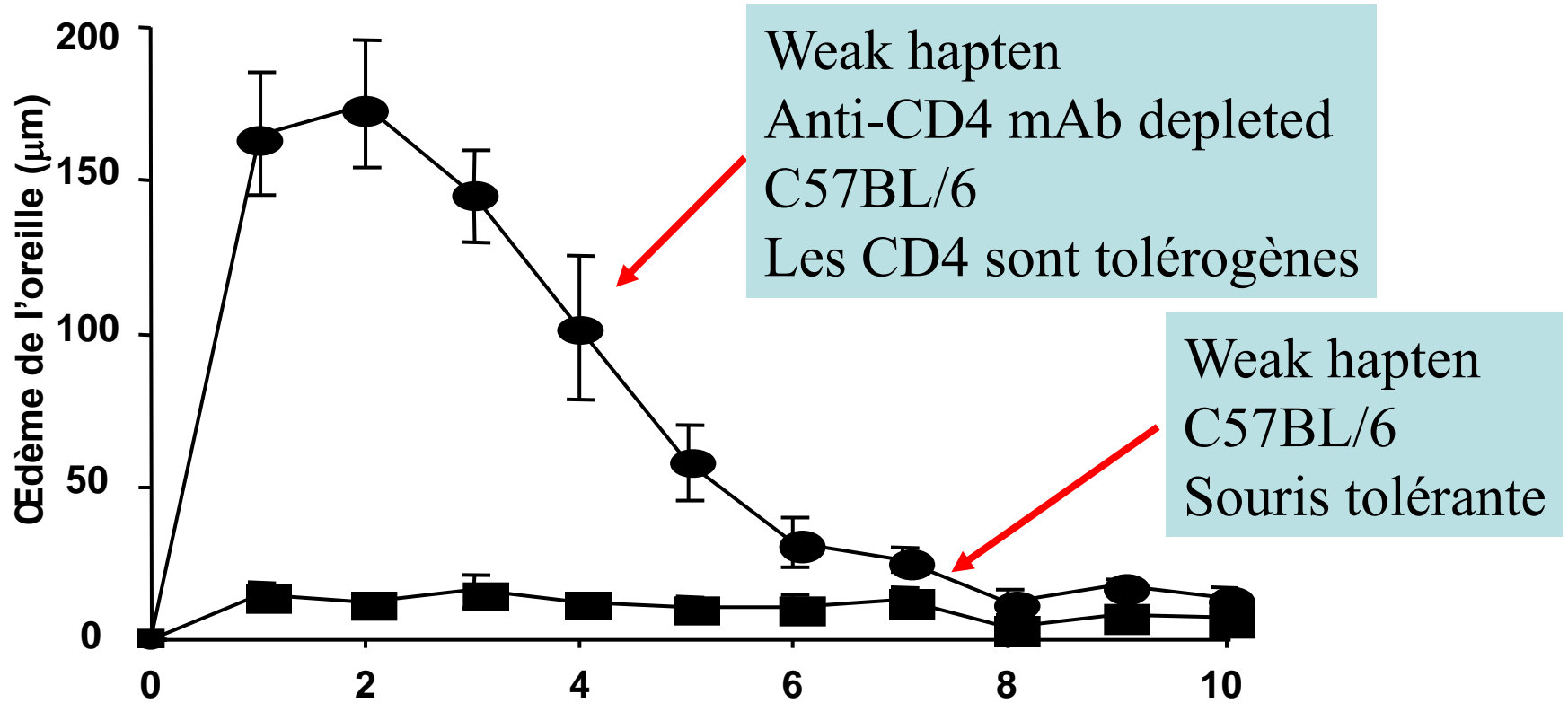
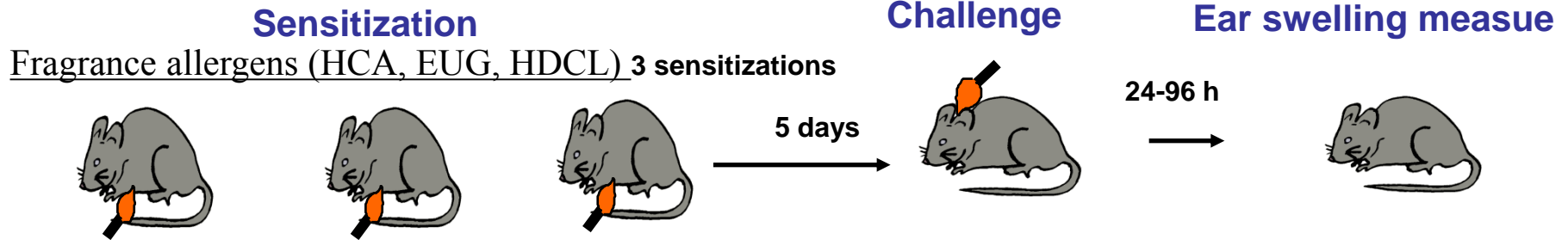
Contact allergy, allergic contact dermatitis or irritant contact dermatitis ?

- Pathophysiology of skin irritation and allergy
- How to differentiate allergy from irritation ?
- How to differentiate allergy to patch test from clinically relevant allergy ?
- Exposure to allergens promotes tolerance

SKIN INFLAMMATION ALLERGY OR IRRITATION ?

- Pathophysiology of skin irritation and allergy
- How to differentiate allergy from irritation ?
- How to differentiate allergy to patch test from clinically relevant allergy ?
- Exposure to allergens promotes tolerance

Allergic contact dermatitis - Weak Haptens



Skin Contact Irritation Conditions the Development and Severity of Allergic Contact Dermatitis

Marlene Bonneville¹, Cyril Chavagnac¹, Marc Vocanson¹, Aurore Rozieres¹, Josette Benetiere¹, Ingrid Pemet², Alain Denis², Jean-Francois Nicolas^{1,3,4} and Ana Hennino¹

Irritant contact dermatitis (ICD) is a frequent inflammatory skin disease induced by skin contact with low molecular weight chemicals such as haptens endowed with proinflammatory properties. Allergic contact dermatitis (ACD) is a frequent complication of ICD and is mediated by hapten-specific T cells primed in lymph nodes by skin emigrating dendritic cells. The aim of this study was to analyze the relationship between ICD and ACD to 2,4-dinitrofluorobenzene (DNFB) in C57BL/6 and BALB/C mice, which develop a severe and a moderate skin inflammation, respectively. Upon a single skin painting with DNFB, C57BL/6 developed within hours a more severe dose-dependent ICD response as compared to BALB/C mice, which was associated with enhanced upregulation of IL-1 β , IL-6, and IL-10. Skin exposure to a low dose of DNFB resulted, in both strains, in a low ICD that resolved in a few hours. Alternatively, skin painting with either an intermediate or a high DNFB concentration induced an ICD that subsequently gave rise to an ACD reaction whose intensity was proportional to the magnitude of the ICD response and was more severe in C57BL/6 mice than in BALB/C mice. In conclusion, the hapten-induced skin contact irritation conditions the development and the severity of ACD.

Skin irritation conditions the severity of ACD

« L'irritation cutanée fait le lit de l'allergie »

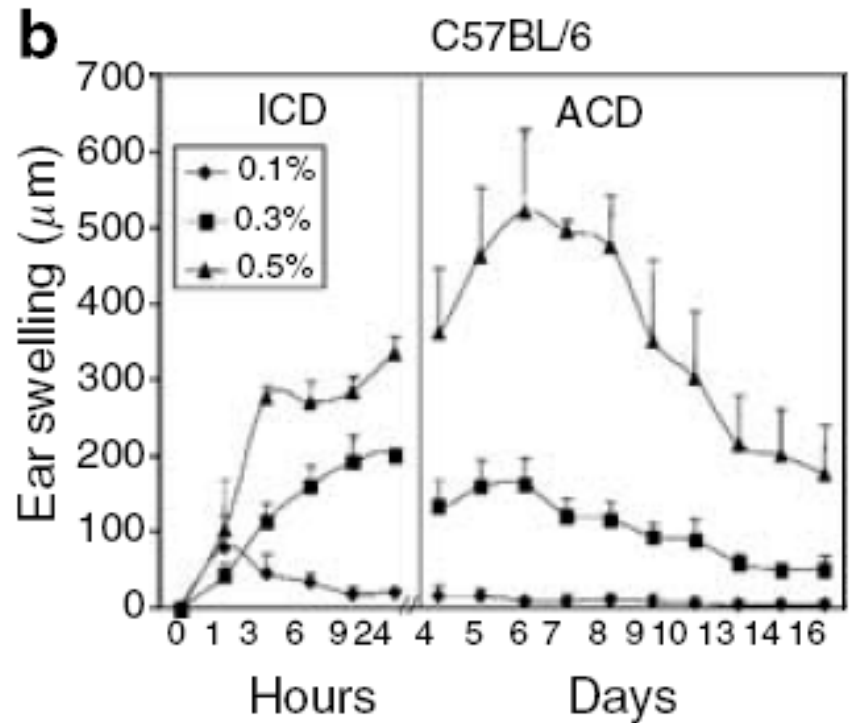
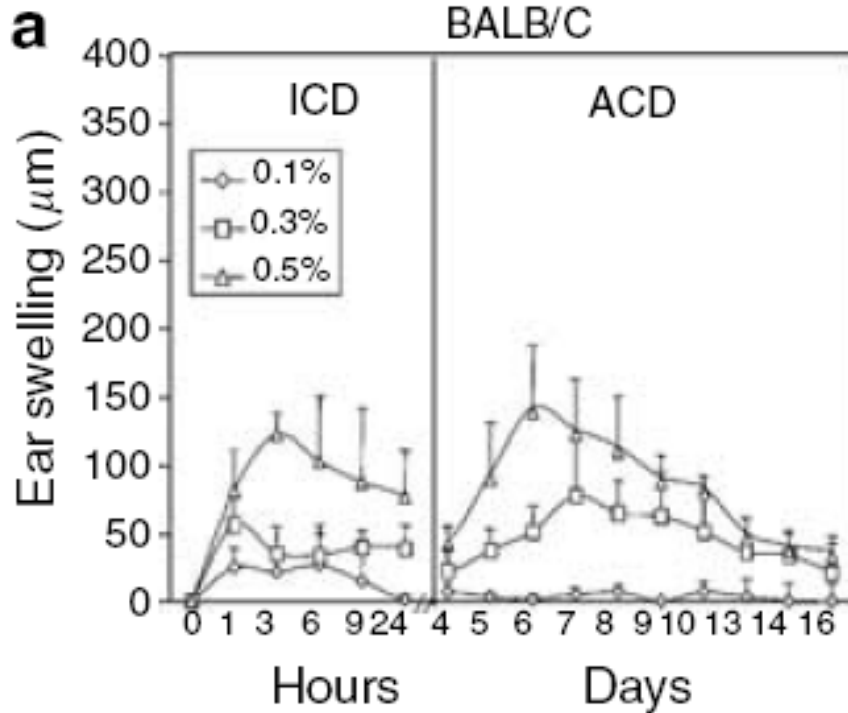
DNFB sur l'oreille
gauche
J0



Mesure de l'œdème
de l'oreille
H0 H3 H6 H9 H24



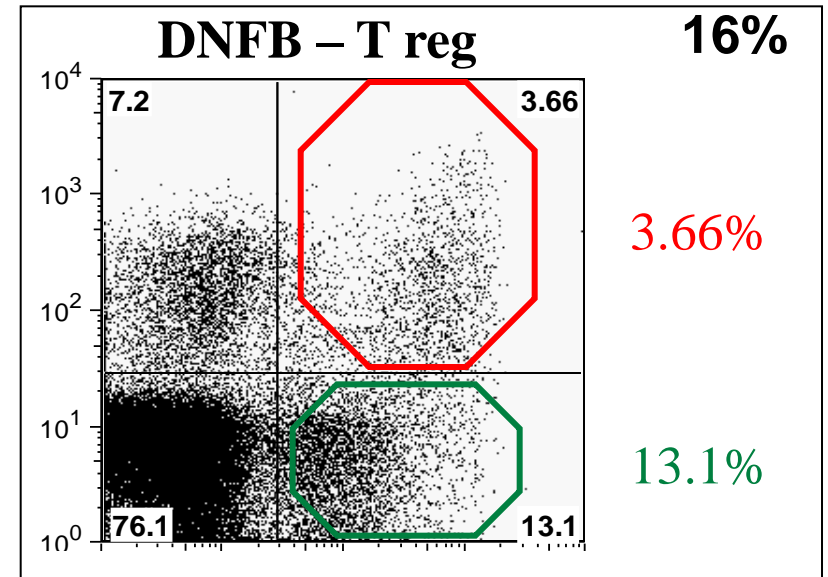
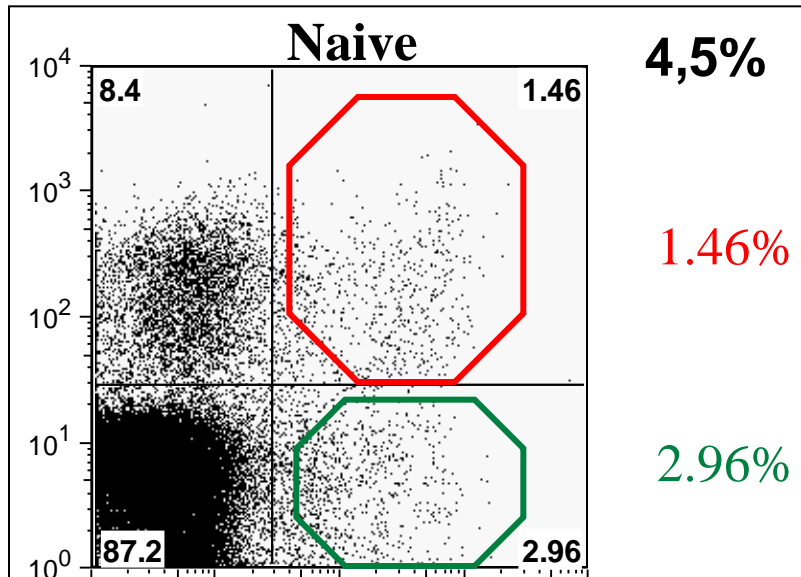
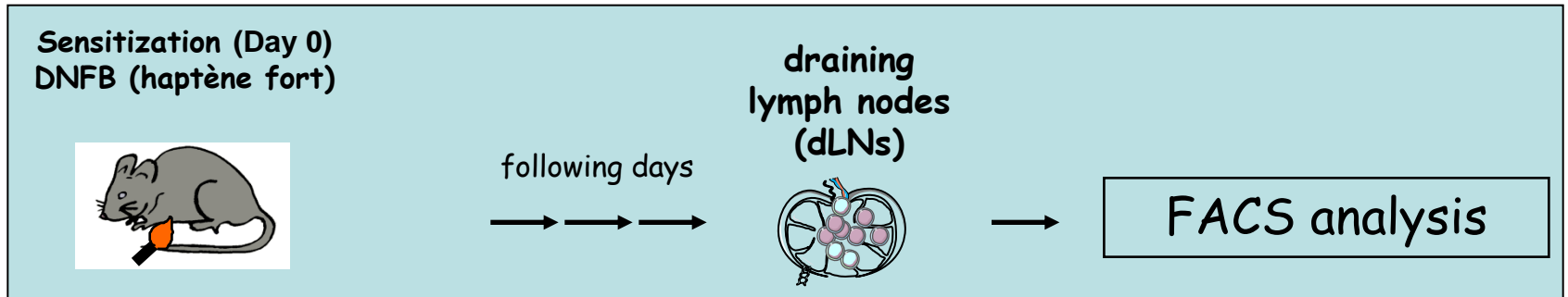
Mesure de l'œdème
de l'oreille
J5/J10



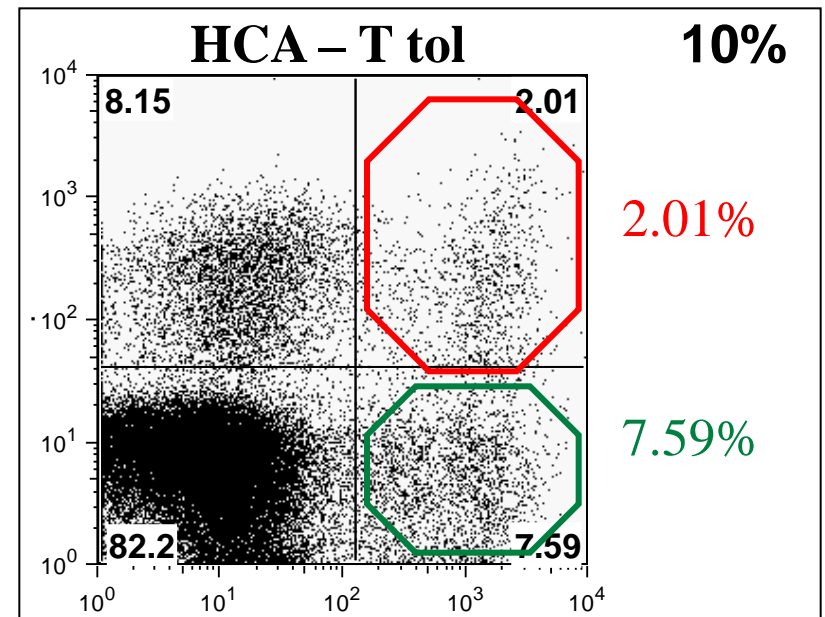
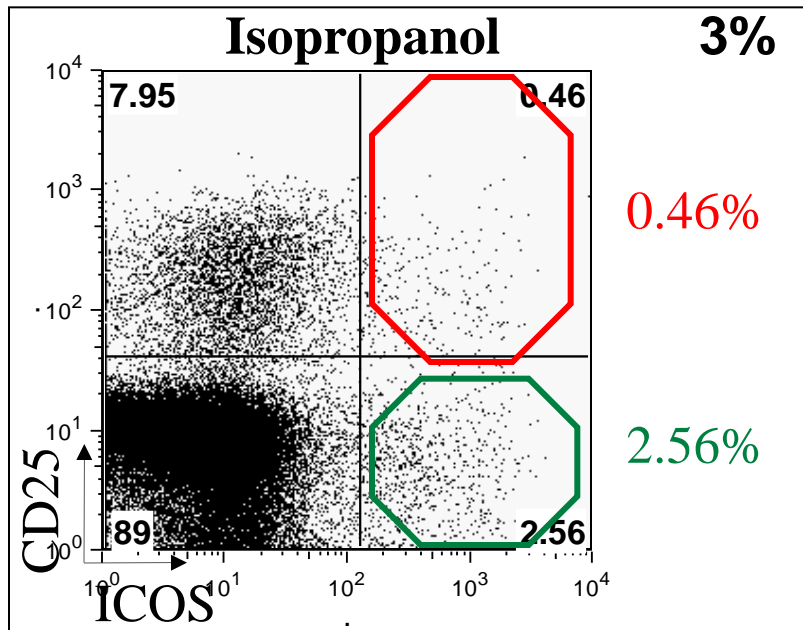
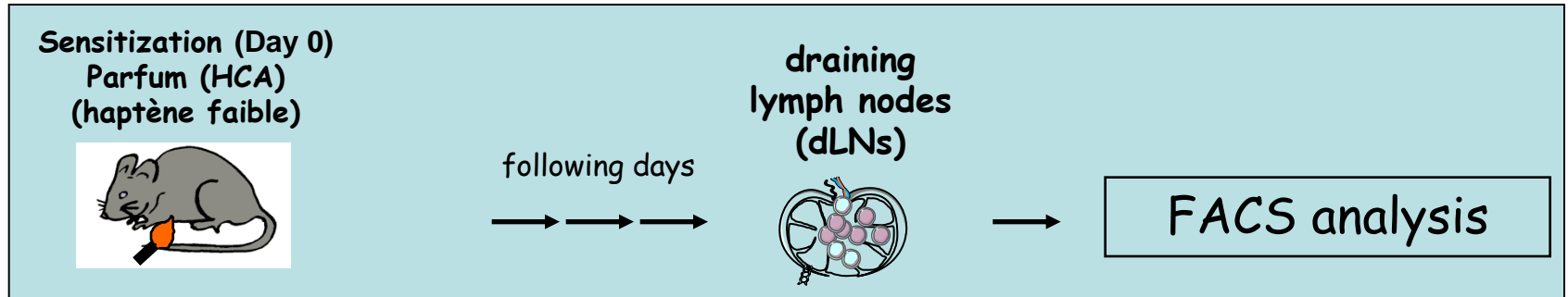
SKIN INFLAMMATION ALLERGY OR IRRITATION ?

- Pathophysiology of skin irritation and allergy
- How to differentiate allergy from irritation ?
- How to differentiate allergy to patch test from clinically relevant allergy ?
- **Exposure to allergens promotes tolerance**

Les haptènes forts activent fortement les LT CD4+ reg qui limitent l'inflammation de l'EAC



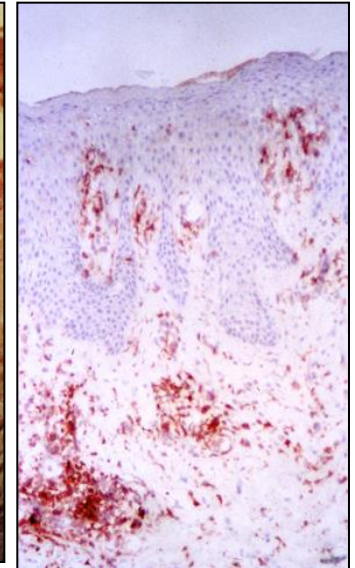
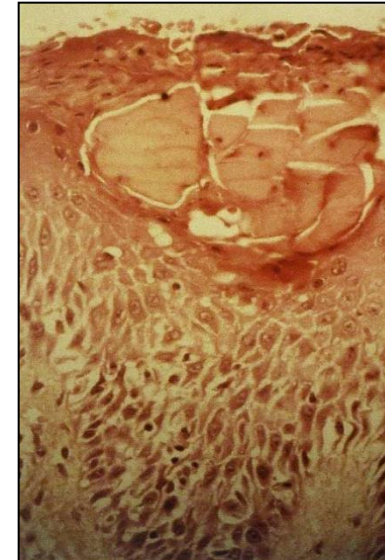
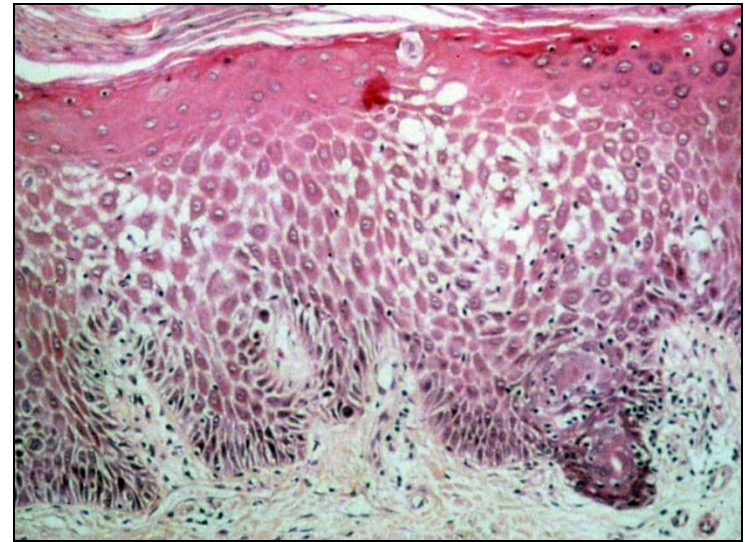
Les haptènes faibles (parfums) activent fortement les LT CD4+ reg/tol qui préviennent l'immunisation et donc qui empêchent la survenue de l'EAC

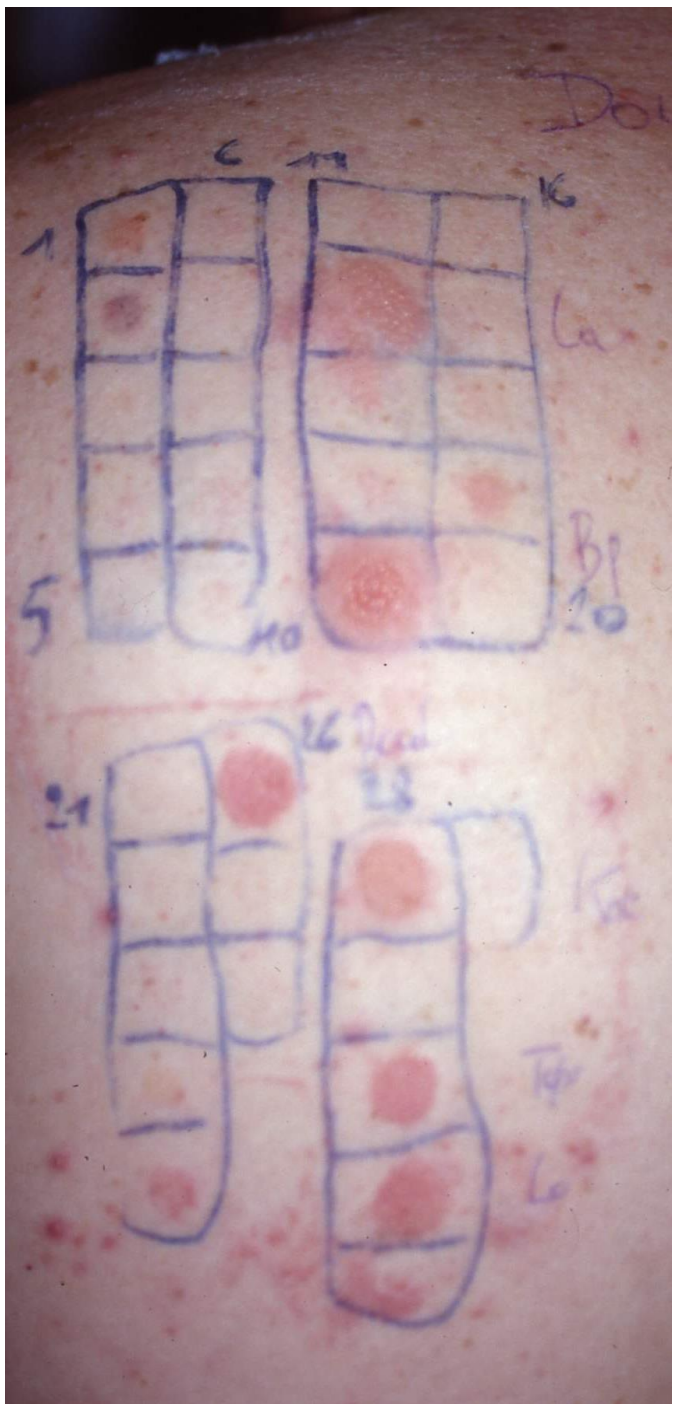


SKIN INFLAMMATION ALLERGY OR IRRITATION ?

- Pathophysiology of skin irritation and allergy
- How to differentiate allergy from irritation ?
- How to differentiate allergy to patch test from clinically relevant allergy ?
- Exposure to allergens promotes tolerance

Irritant versus Allergic Contact Dermatitis





How to differentiate irritation from allergy?

- 10 positive patch tests
- Are there irritant or allergic ?
- Are true positive tests relevant ?

By demonstrating the existence of an allergic reaction

1. Modify the patch testing protocol

- Patch-test applied for 24 hrs (12 hrs)
- Reading at 48/72 hrs

2. Characterize the skin DTH reaction: biopsy

- T cell infiltration
- T cell activation

3. Characterize circulating specific T cells

- LTT
- Elispot

Strong and weak haptens

Nom	Source	Pouvoir sensibilisant
2,4-Dinitrochlorobenzène	Chimie	Fort
2,4-Dinitrofluorobenzène	Chimie	Strong/Fort
2,4-Dinitroiodobenzène	Chimie	Fort
Disperses Blue 124 ou 106	Textile (Colorants)	Faible
Dichromate de potassium	Bâtiment (ciment)	Modéré
Sulfate de nickel	Bâtiment, Bijoux fantaisie	Modéré
Formaldéhyde	Cosmétique, Textiles, Désinfectant	Faible
Glutaraldéhyde	Conservateur, Antiseptique	Faible
p- Phénylénédiamine	Cosmétique (colorant capillaire)	Faible
Eugénol	Cosmétique, Antiseptique	Weak/Faible
Hexylcinnamique aldéhyde	Cosmétique (parfum)	Weak/Faible
Hydroxycitronellal	Cosmétique (parfum)	Weak/Faible
Amoxicilline, Cyanamide, Cetrimide...	Médicament	Weak/Faible

DRUG ALLERGY - INTRODUCTION



Mortality

Prevalence

- TEN: Toxic Epidermal Necrolysis
- DRESS: Drug Rash with Eosinophilia and Systemic symptoms
- AGEP: Acute Generalized Exanthematous Pustulosis
- FDE: Fixed Drug Eruption
- Erythema multiform
- Others severe reactions



**Drug allergy – DTH reactions or not ?
What is the offending drug ?**

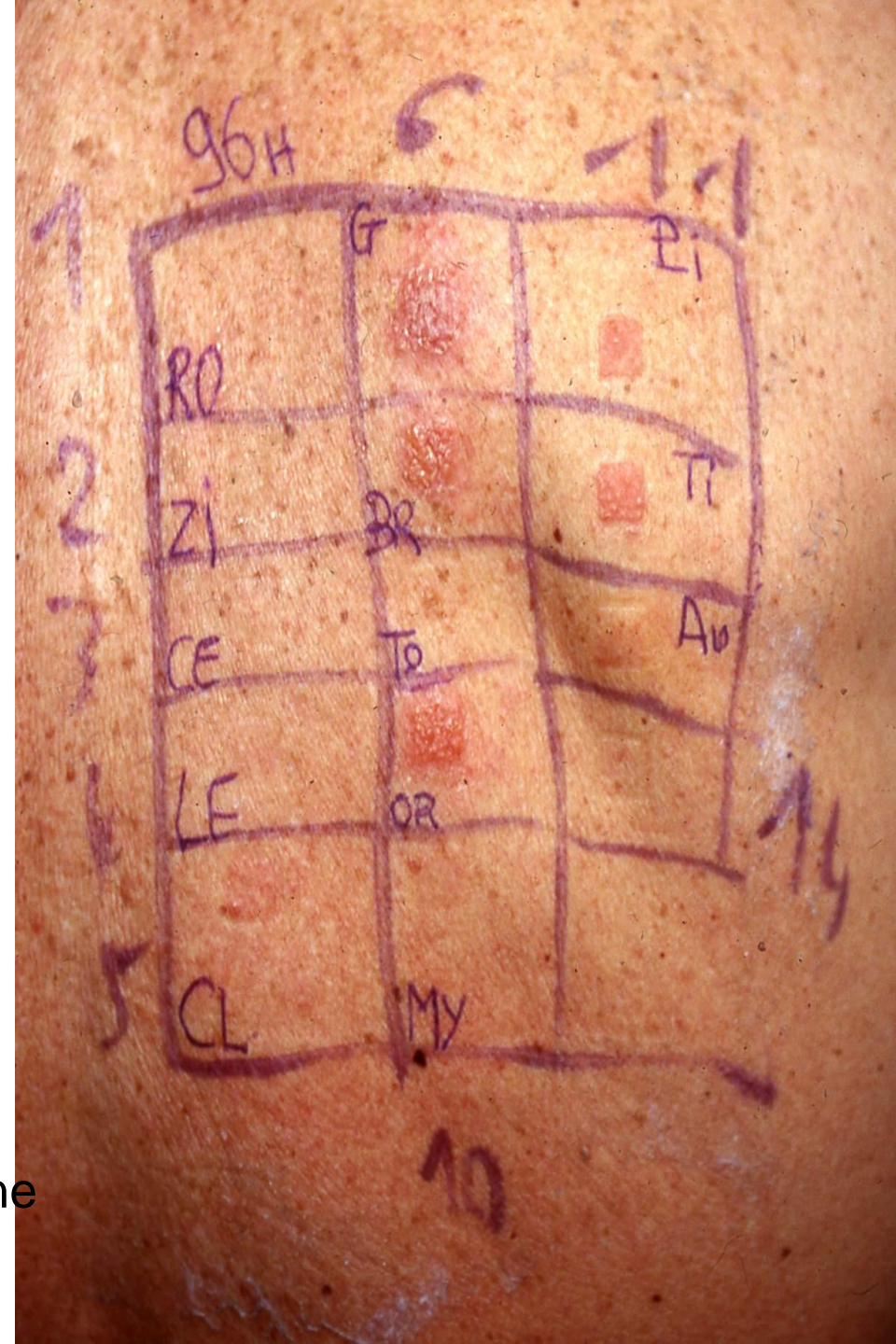


Maculo-papular exanthema Clamoxyl (peni A)



MPE after clamoxyl + ibuprofene
replaced by sulfamethoxazole +
tenoxicam





MPE to peni M (Bristopen) + Oropivalone + ketoprofene + paracetamol

FIRST PATCH TESTING – Allergic and irritant positive patch tests

Mr V.

Drug-induced maculo-papular exanthema

January 2004 – 5 days after a
prostatectomy + GA

1st testing (patch removal 72h):

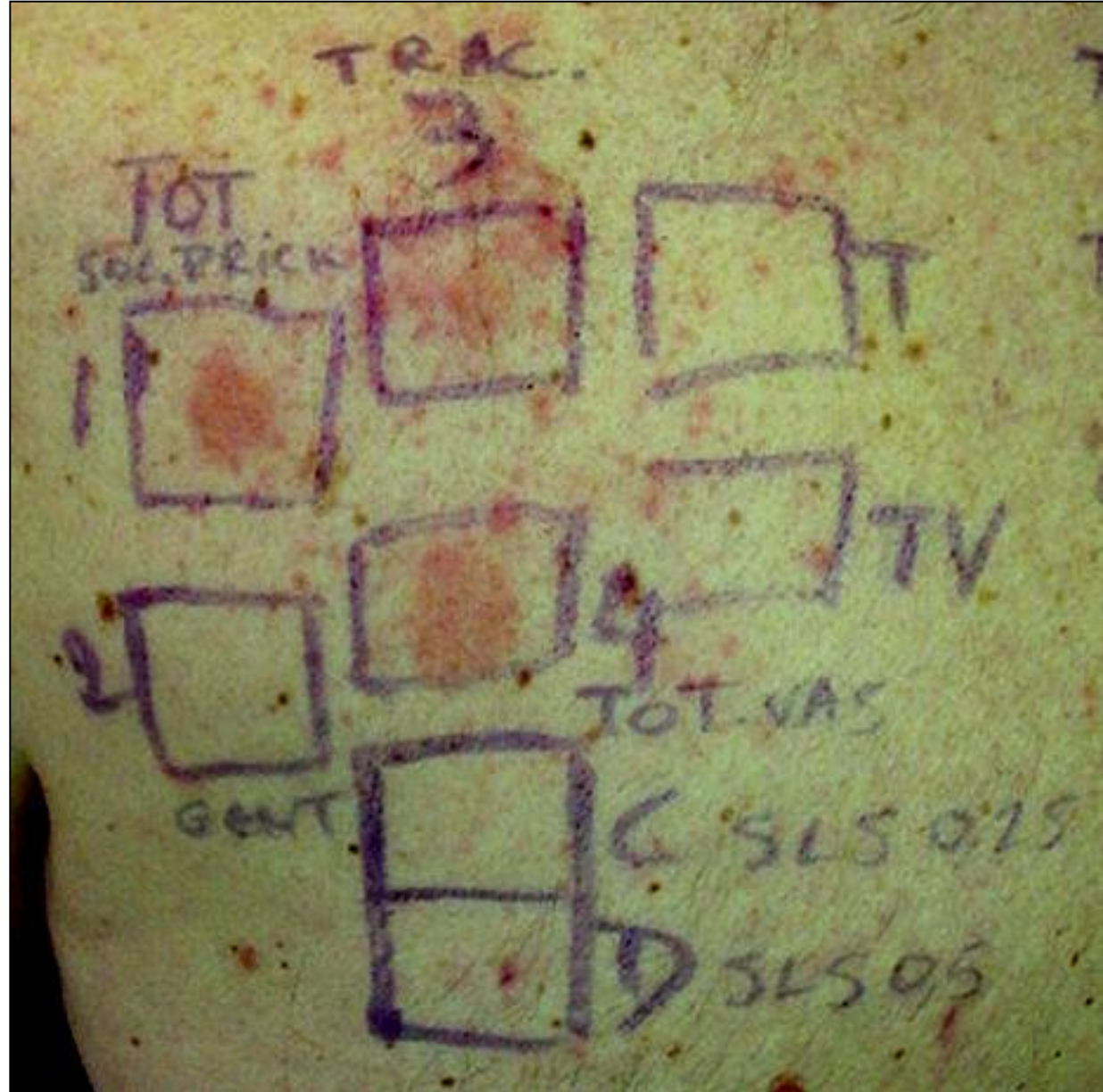
Tracrium +
Ampicillin (IV solution + petrolatum)
Gentamycin +
SLS 0,5 et 0,25+

- Several positive patch tests
- Irritation control (SLS) positive
- No conclusion



SECOND PATCH TESTING – Early removal of patch tests decreases the irritation properties of chemicals

FIRST PATCH TESTING



Patch removal at 12 hours

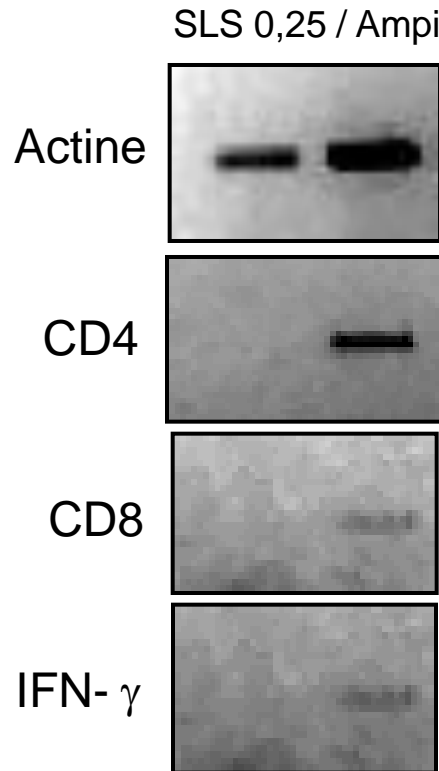
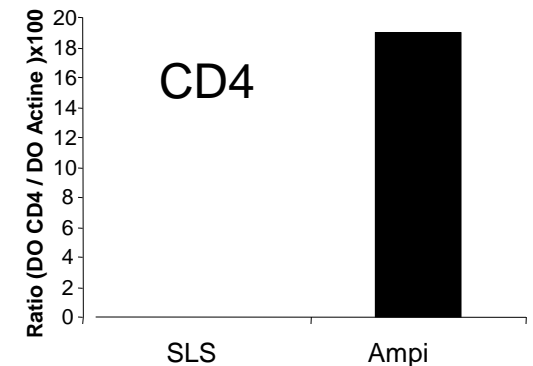
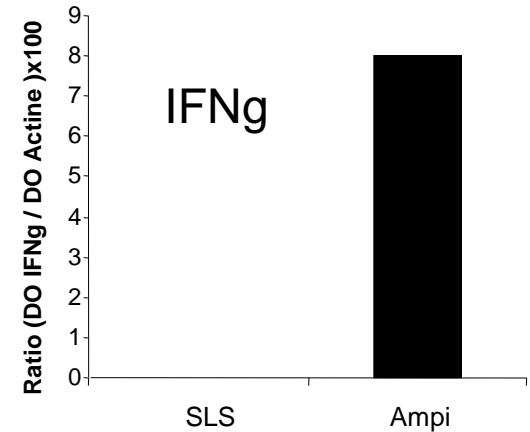
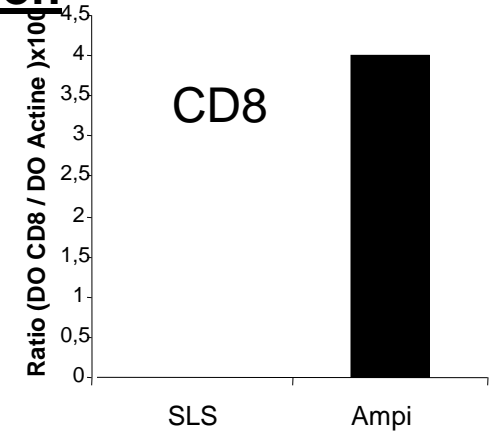
- Ampicillin solution ++
- Ampicillin petrolatum ++
- Tacrium patch: -
- Gentamycin patch: -
- SLS 0.25:-
- SLS 0.5:-

SKIN BIOPSIES – Presence of T cells infiltrating the patch tests

RT-PCR analysis of T cell infiltration and T cell activation

Skin Biopsies (4mm diameter). Deep frozen
RNA extraction
Probes for CD4, CD8 and IFN γ (cDNA)
Semi-quantitative RT-PCR

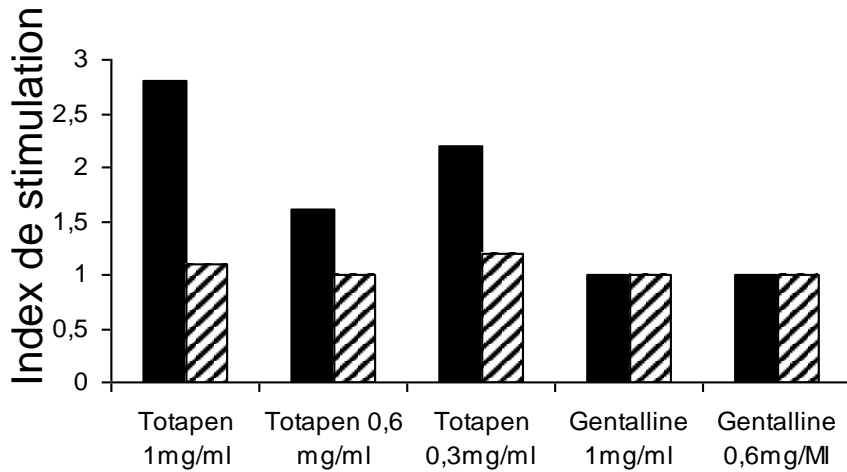
Results: T cell infiltration/activation found only in ampicillin positive patch test



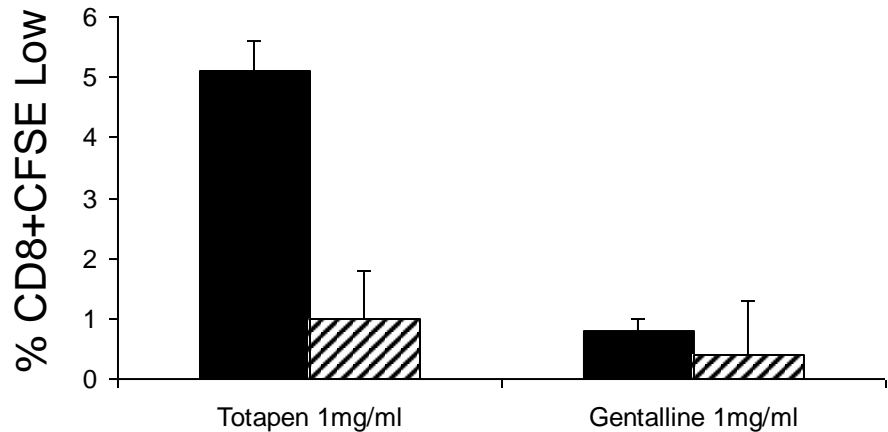
BLOOD - Presence of hapten-specific T cells

Detection and enumeration of hapten-specific T cells in blood

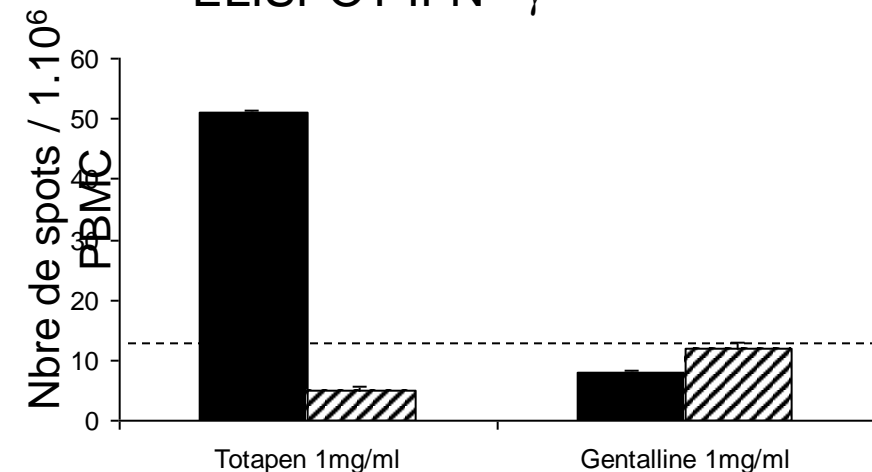
LTT:



CFSE



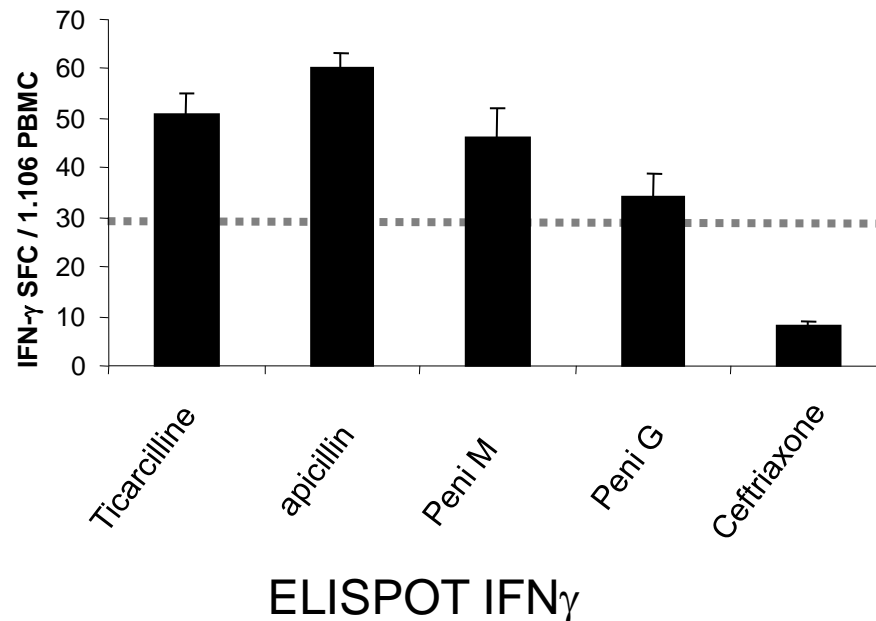
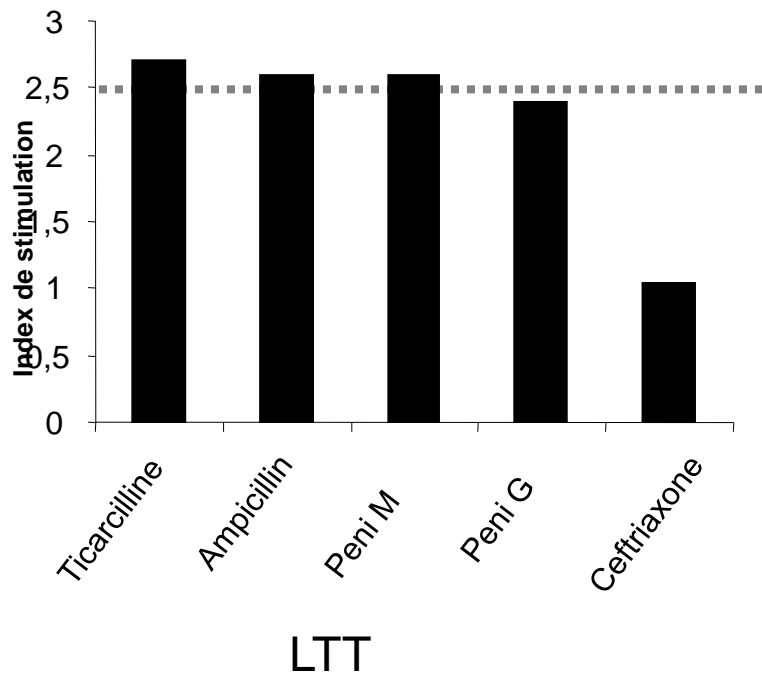
ELISPOT IFN- γ



- LTT positive ampicillin SI=2,8 /
- CD8+CFSE low ampicillin 5,1%
- ELISPOT positive ampicillin 52 spots / 1.10⁶ PBMC
- Tests negative for gentamycin

CONCLUSION: presence of ampicillin-specific T cells in blood

BLOOD – Characterization of T cell cross-reactivities to different but related chemicals



⇒ **Cross-reactivity between ticarcillin, peni A, peni M, peni G:**
These antibiotics are contra-indicated

⇒ **No cross-reactivity with ceftriaxon (Rocephine®)**

SKIN INFLAMMATION ALLERGY OR IRRITATION ?

- Pathophysiology of skin irritation and allergy
- How to differentiate allergy from irritation ?
- How to differentiate allergy to patch test from clinically relevant allergy ?
- Exposure to allergens promotes tolerance

- False + patch-tests
- Sensitization versus allergy
- Clinical relevance of skin tests
- Open tests et ROAT

Allergic contact dermatitis from povidone-iodine: a re-evaluation study

JEAN-MARIE LACHAPELLE

Department of Dermatology, Louvain University, 30, Clos Chapelle-aux-Champs, UCL 3033,
B-1200 Brussels, Belgium

500 consecutive patients were patch tested with a 10% povidone-iodine (PVP-I) solution, diluted 10 times in water. Readings were made at 2 and 4 days. 14 of the 500 (2.8%) showed a positive test to PVP-I. The 14 positive patients to PVP-I were subjected to a repeated open application test (ROAT) with a PVP-I solution, as is; 2 of the 14 were positive. The 12 negative patients were tested with 500 patients had true allergic contact dermatitis. The 12 patients were tested for more complete investigation, when tested with irritants and allergenic properties, such as PVP-I. The results showed that the reactions were irritant reactions and to assess true clinical relevance.

Key words: allergic contact dermatitis; patch test; ROAT
© Blackwell Munksgaard, 2005.

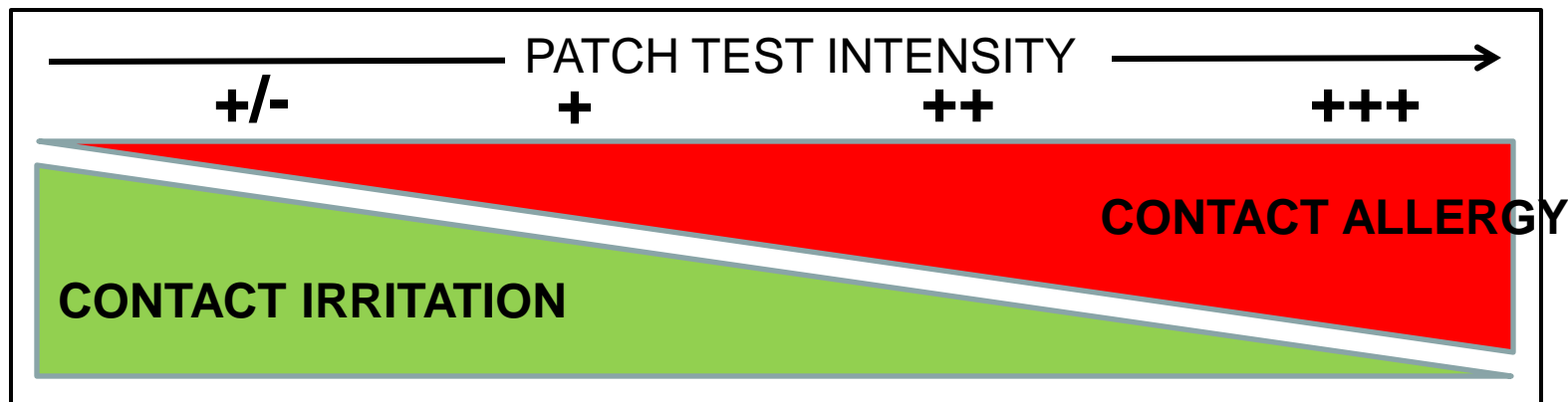
12/14 patch^{pos}, roat^{neg} ?

- irritants

- patch^{pos} non pertinents

Contact Allergy to fragrances

- Prevalence of fragrance contact allergy: 7%
- Positive patch-test = Fragrance-induced skin inflammation
 - Innate immunity: contact irritation (inflammasome; IL-1 & other primary cytokines)
 - Adaptive immunity: contact allergy (T cell- mediated, CD8+ T cells, IFN γ)
- What is the real prevalence of fragrance contact allergy ?
 - Which proportion of positive tests are true positive and represent allergic tests?
 - Which proportion of positive tests are false positive and represent irritative tests?
 - Open tests and repeated open tests (ROAT) are needed for diagnosis



Contact Dermatitis to fragrances

- Clinical lesion of eczema
- Skin inflammation
 - Innate immunity: irritant contact dermatitis (inflammasome; IL-1 & other primary cytokines)
 - Adaptive immunity: allergic contact dermatitis (T cell-mediated, CD8+ T cells, IFN γ)
- Prevalence of CD to fragrances unknown
 - No strong epidemiological data
 - No cosmetovigilance data
- Prevalence of ACD to fragrances unknown
Misinterpretation:
 - Irritant contact dermatitis
 - Atopic dermatitis (facial eczema, eyelid eczema)
- Prevalence of ACD to fragrances probably very low (example though the coumarine studies)
- Studies needed on ACD to fragrances and not on contact allergy to fragrances: epidemiology, cosmetovigilance, immunology etc..

ALLERGIC CONTACT DERMATITIS

Contact Hypersensitivity



Question 1: IS THIS AN ECZEMA/DERMATITIS ?

Question 2: IS THIS A CONTACT DERMATITIS ?

Question 2: IS THIS AN ALLERGIC CD ?

ALLERGIC CONTACT DERMATITIS

Contact Hypersensitivity



**Question 1: irritation (false +)
or allergy (real +) ?**

- irritation: non-specific inflammation
- allergy: specific, T cell-mediated, inflammation

**Question 2: Is the real + test
clinically relevant ?**

- sensitization, DTH, chemical- specific T cells
- allergy to patch test
- patch testing is a maximization test and real+ patch means the existence of specific T cells
- real + patch test does not mean that these T cells could be activated in normal conditions of exposure to the chemical