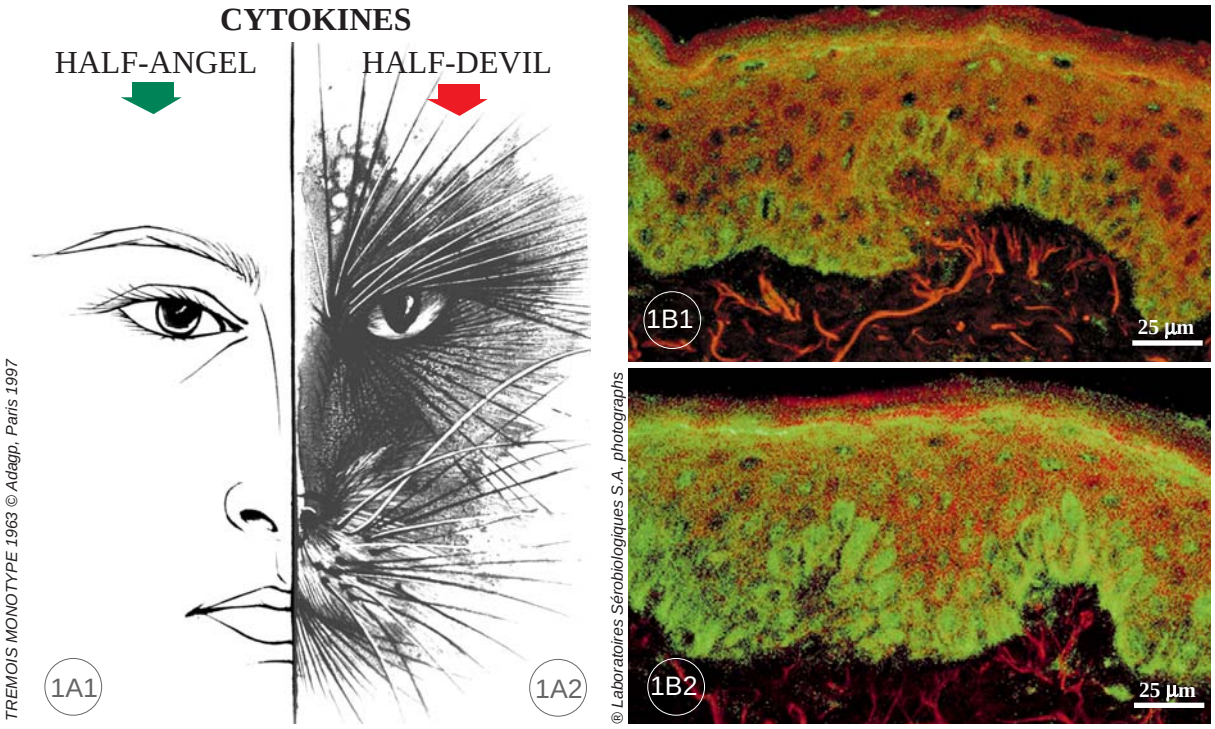
• *Cytokine-like* factor of intercellular communication
• Dynamizing agent of skin regeneration and protection



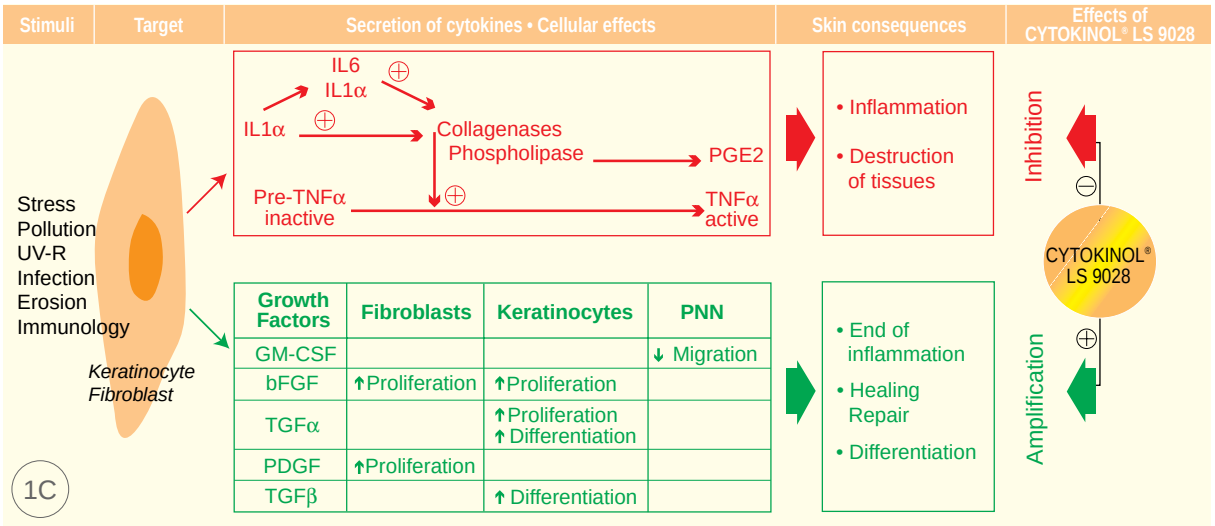


Fig 1 – Cytokines*: biomolecules supervising the universal language in the dialogue between cells, related to physiological processes.*
1B *- UV-R, a stimulus among others, induces an epidermal invasion by interleukin IL-1α (immunohistochemical visualization, green color: irradiated skin* ***1B2****, versus control skin* ***1B1*** *(Laboratoires Sérobiologiques S.A. photographs).*
1C *- At the skin level, various stimuli induce: the IL-1α - TNFα release, starting inflammation and tissue necrosis (1A2 devil's side) and the release of growth factors (GF) accelerating healing and differentiation (1A1 angel's side).*
CYTOKINOL®* LS 9028*, a cytokine-like peptidic mediator, has both effects: GF like amplification, regulating speed and quality of skin repair, and IL-1α inhibition, strengthening the cutaneous potential of defense (against stress, pollution, UV...).**

Cytokines and skin

• Cells, tissues and organs communicate with each other via mediators enabling them to keep a homeostasis, or to react on exogenic or endogenous attacks. The main mediators can be classifyed in: hormones, neuropeptides, lipidic metabolites, saccharides, nucleotides, amino-acids, ions and cytokines.

• **Cytokines are polypeptides of intercellular communication** (from 5 to 80 kDa) released by many activated cellular populations, and playing a role in: immune response, inflammation, maturation and cellular differentiation, synthesis of macromolecules within the extracellular matrix.

• Cytokines **act on target cells** through some specific receptors on the membrane of these cells.

• Cytokines are **divided into groups**:

1. Interferons (IFN) anti infectious and anti tumoral (secreted by monocytes, Langerhans cells),
2. Colony Stimulating Factors (CSF) amplify the antigen-presenting function of Langerhans cells,
3. Tumor Necrosis Factor (TNFα) released by macrophages; stimulation of immunity and inflammation.
4. Anticytokines or natural inhibitors of Il-1, TNFα,
5. Growth Factors (GF) and Transforming Growth Factor (TGF) PDGF, IGF, FGF, EGF have an anabolic and regenerating function (healing accelerators). TGF: inhibitor of cellular proliferation and cytokines, stimulator of collagen synthesis,
6. Interleukin (Il-1α): released by macrophages, it acts on lymphocytes and cells of connective tissue, induces the proliferation of fibroblasts. It can also be released by Keratinocytes as an inflammatory reaction.

Other Il: 2, 3, 4, 5, 6, 7, 9, 10 and 11.

• The **role of cytokines** at the skin level is essential. They are secreted by epidermal and dermal cells (keratinocytes, dendritic cells, fibroblasts…). The targets of cytokines are multiple; according to the target cell and the concerned cytokine, the induced effect deals with various physiological mechanisms: regulation of cellular differentiation, cellular growth, keratinization or healing/repair process.

This interactive system is also interesting against inflammatory reactions, defense against infectious agents, tumors and environmental attacks. Cytokines are strong meditors used in therapeutics; they can have many side effects.

CYTOKINOL® LS 9028

Concept

• In cosmetology, the use of therapeutic proteins, especially cytokines, cannot be considered, as they are drugs with some possible side effects.

• However, the concept of cytokine-like mediators is quite attractive. Our Laboratories have orientated their research towards new active substances, free of any side effects, having a chemical structure being close to cytokines (peptides).

These substances should have both, a cytokine like good effect (pro-angel) and an anti-cytokine effect, in order to fight against the negative side of their activities (anti-devil).

CYTOKINOL® LS 9028 is the concrete result of this concept.

Definition / Composition

• CYTOKINOL® LS 9028 is a unique, concentrated complex of peptides having a mixed (lactic and yeast) origin, combined with one specific amino-acid. CYTOKINOL® LS 9028 is a cytokine-like "messenger" because of:

- some homology with cytokines (EGF, FGF, IL),
- modulating benefits: activating of the EGF, FGF type and inhibiting of IL1-α hypersecretion…

Reminder: other casein peptides, β-casomorphines are known for their activities: opiate, immunostimulating, anti-hypertensive and antithrombotic ones.

Main components:

	MW	%
• Poly- and oligo-peptides		
- of *lactic* origin	5 to 30 kDa	~ 22
- of *yeast* origin	≤ to 5 kDa	~ 78

• L-Amino-acid = Lysine

Skin benefits

1. At the **epidermal** level:
- **factor of** keratinocyte **activity** and **growth**,
- **stimulant** of keratinocyte migration and epidermization,
- stimulant of **epidermal differentiation** and **ortho-keratinization,**
- strong epidermal **healing** and **repairing effect**.

2. At the **dermal** level:
- stimulant of fibroblast activity,
- strengthening the **contraction** and **structuring** of the extracellular matrix.

3. On the **whole skin**:
- strengthening the **resistance** against environmental **attacks**: anti UV-R, pollutants, irritants, sensibilizers; soothing, **local anti-inflammatory**,
- firming, **structuring** and **tightening**,
- softening, **toning** and **elasticizing**.

Cosmetic use

- Care of **devitalized, dull, atonic, aged** skin.
- **Repairing** and **dynamizing** care of **tired skin, with a lack of firmness**.
- **Anti-stress**, **anti-attack** protecting care.
- Care for **sensitive skin**.
- **Rejuvenating** care: helping the **structuring** and the **quality of the skin**.
- Face, body and hand care; sun care; men care; local slimming care.

Dosage / Solubility / Mode of incorporation

1. Dose of use: 0.50% to 1%.

2. Solubility: hydrosoluble, insoluble in oils.

3. Mode of incorporation: prepare a fresh aqueous mother solution with 20% of CYTOKINOL® LS 9028; then disperse it into the cosmetic preparation during the finishing process.

Analytical characteristics

1. Aspect: a white fine powder, with weak odor.

2. Specifications: upon request.

Tolerance

Good.

Efficacy

Test summaries overleaf.

Storage

In its original packaging, at 15-25°C.

INCI Name

Hydrolyzed Casein (and) Hydrolyzed Yeast Protein (and) Lysine HCl.

Manufacturer

Laboratoires Sérobiologiques S.A.

Stimulation of the proliferation and the migration of human epidermal keratinocytes, in vitro.

Aim

Determination of stimulating potential of CYTOKINOL® LS 9028 on keratinocyte growth and differentiation.

Protocol

Keratinocytes have been inoculated in a small cylinder on a Petri box coated with collagen. When keratinocytes are fixed, the cylinders are removed and the initial culture medium is replaced with media to be tested. Foetal Calf Serum (FCS) has been used as the standard reference as it contains many cytokines (EGF, FGF, CSF…). After 7 and 14 days, the new formed epidermis has been fixed, stained and quantified by image analysis.

Results (Fig. 2 and 3)

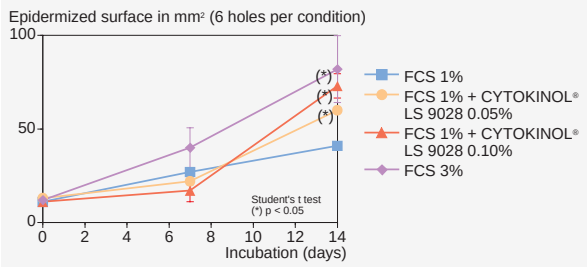


***Fig. 2** – CYTOKINOL® LS 9028: activating effect of epidermization.*

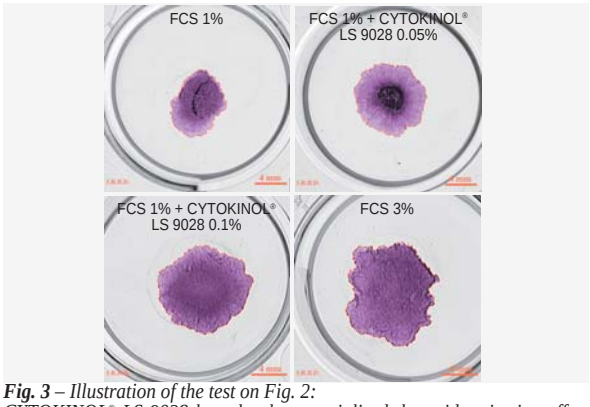


***Fig. 3** – Illustration of the test on Fig. 2: CYTOKINOL® LS 9028 has clearly potentialized the epidermization effect of FCS; thus, CYTOKINOL® LS 9028 at 0.10% + FCS at 1%, has nearly the same effect as FCS alone at 3%.*

Stimulation of the proliferation and the structuration of human fibroblasts, in vitro.

Aim

Determination of activating potential of CYTOKINOL® LS 9028 on fibroblast growth and structuring activity.

Protocol

MRC5 fibroblasts were incubated with CYTOKINOL® LS 9028 for 10 days on a collagen lattice. The surface of this lattice was measured daily.

Results

CYTOKINOL® LS 9028 is a (peptidic) bio-messenger with a cytokine like action, able to induce at a very low concentration (0.10%):

- a clear proliferation of fibroblasts: +29% ($p < 0.05$) after 7 days of incubation,
- a strong structuring, toning, strengthening effect on the dermal extracellular matrix by fibroblasts (Fig. 4).

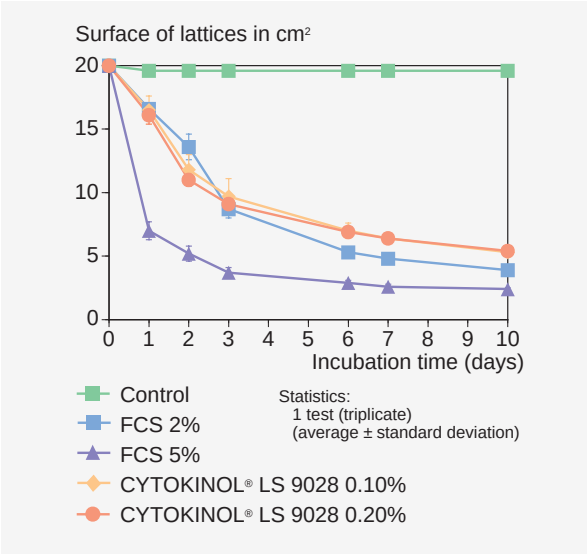


***Fig. 4** – Effect of CYTOKINOL® LS 9028 on the structuration of the dermal extracellular matrix by fibroblasts. Contraction of the lattice within 10 days. Results after 3 days: CYTOKINOL® LS 9028 at 0.10% has stimulated by 45% the contraction (effect is equivalent to FCS 2% alone).*

Inhibition of IL-1α, UV induced, on human keratinocytes, in vitro.

Aim

Determination of the anti-inflammatory activity of CYTOKINOL® LS 9028. Measurement of IL-1α release.

Protocol

The induction of PGE2 and IL-1α has been carried out on cultures of human keratinocytes, irradiated by UV-B, in presence of increasing concentrations of CYTOKINOL® LS 9028, versus control.

Results

• After UV-R irradiation on keratinocytes, the PGE2 level has been multiplied by 50. CYTOKINOL® LS 9028 at 0.40% has decreased by 90% PGE2 ($p < 0.05$). (Fig. 5)

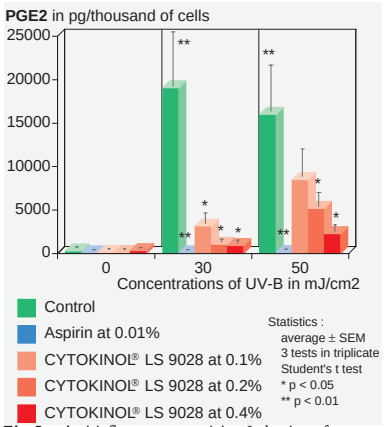


***Fig. 5** – Anti-inflammatory activity. Induction of prostaglandins (PGE2) by UV-B on human keratinocytes (NCTC 2544). Inhibiting effect of CYTOKINOL® LS 9028.*

• After UV-R irradiation, the IL-1α level released by keratinocytes has been multiplied by 5. CYTOKINOL® LS 9028 at 0.40% has significantly decreased IL-1α (Fig. 6).

CYTOKINOL® LS 9028 is a soothing protector against IL-1α hypersecretion induced by environmental attacks.

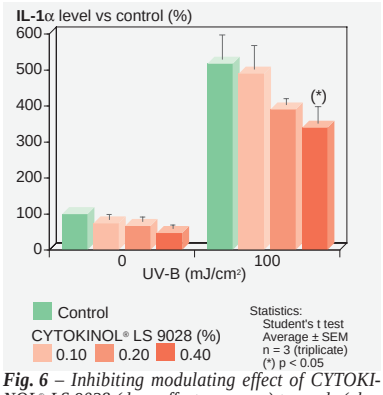


***Fig. 6** – Inhibiting modulating effect of CYTOKINOL® LS 9028 (dose effect response) towards (photoinduced) release of IL-1α by keratinocytes.*

Stimulation of orthokeratotic, epidermal repair, in vivo.

Aim

Evaluation of the structuring potential of CYTOKINOL® LS 9028 on epidermis.

Protocol (Fig. 7)

CYTOKINOL® LS 9028 has been tested at 1% in an emulsion, in topical applications during 2 days, in comparison with the placebo emulsion and the untreated areas (= control).
The repairing activity has been visualized at the epidermal level by histology (HES).
The variations of thickness have been quantified by an image analyzer.

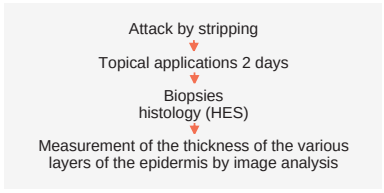


Fig. 7 – Schema of the experimental protocol.

Results (Fig. 8 and 9)

CYTOKINOL® LS 9028 at 1% has clearly stimulated the epidermal repair, not only demonstrated by its quick return to the normal state, but also through the quality of the orthokeratotic type.

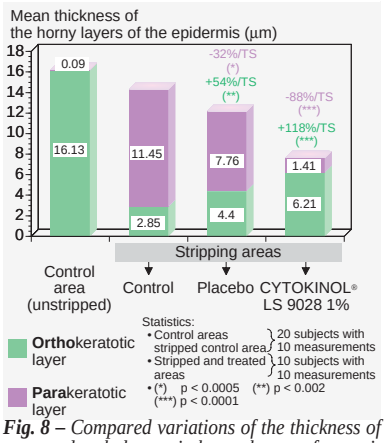


Fig. 8 – Compared variations of the thickness of para and orthokeratotic horny layers after topical applications of the placebo emulsion and CYTOKINOL® LS 9028 at 1%. The improvement induced by the active has been significant compared to the placebo and control areas.

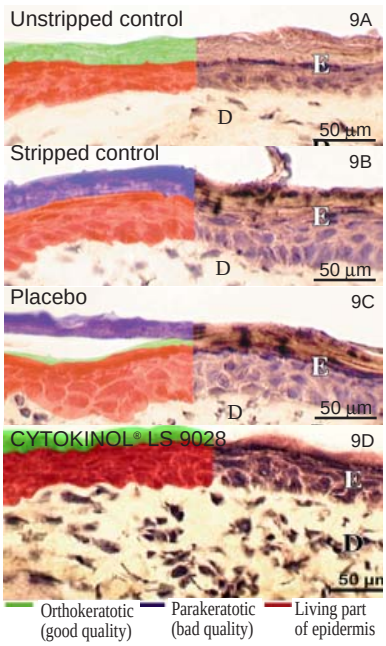


Fig. 9 – Histological illustration of the variations of para and orthokeratotic epidermal horny layers on 4 typical areas: 8A, 8B, 8C and 8D.

Local soothing, protecting effect, IL-1α inhibition after UV-R (clinical test).

Aim

Determination of soothing effect of CYTOKINOL® LS 9028 after UV radiation on volunteers.

Protocol (Fig. 10)

The study has been carried out on 10 healthy volunteers of phototype I to III. The preventive treatment has been randomized in double blind, of standardized topical applications of each of both creams (placebo versus a cream containing 1% of CYTOKINOL® LS 9028) before the induction of the erythema, through a single UV-A + UV-B irradiation with solar xenon simulator.
The local anti-inflammatory effect has been evaluated 24 hours after irradiation:

- by a visual appraisal of the erythema intensity, following a semi-quantitative quotation scale,
- by a quantitative measurement of the erythema intensity, with chromametry, with a calculation of an erythema index based on both chromatic L* and a* coordinates (CIE LAB, 1996).

The area being only irradiated has been used as a comparison control.

Results (Fig. 11)

CYTOKINOL® LS 9028 has shown a clear moderating effect on the UV-R erythema.This effect is significant compared to the placebo and the control area.

Conclusion

A topical use of a cream containing 1%, CYTOKINOL® LS 9028 has clearly improved the potential of skin resistance, against environmental attacks of the UV-R type.

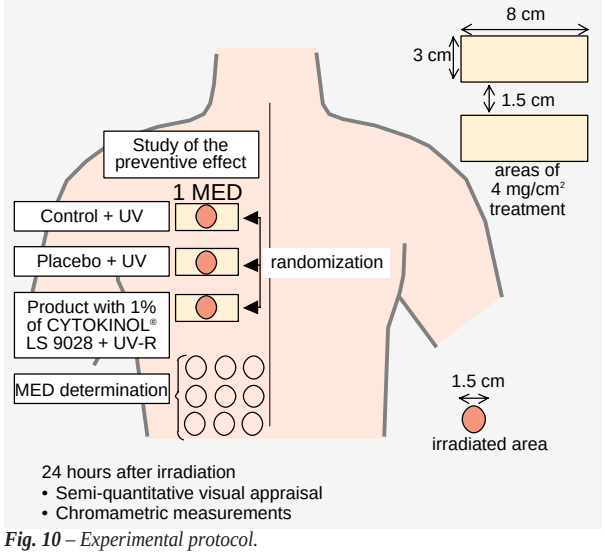


Fig. 10 – Experimental protocol.

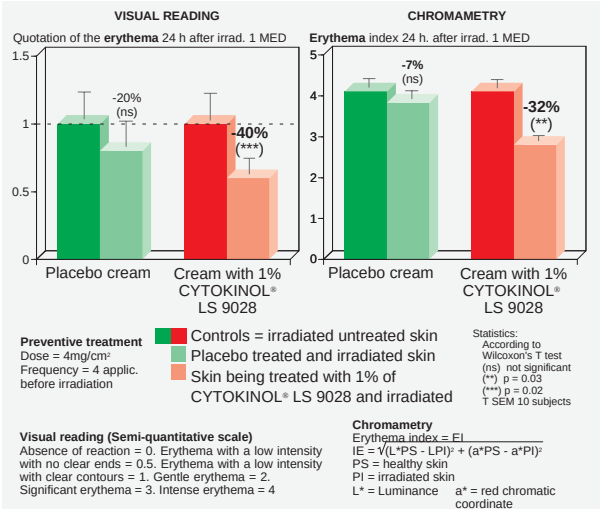


Fig. 11 – Inhibiting effect of CYTOKINOL® LS 9028, against the deleterious effects of UV-R irradiation. Quantification with 2 techniques .

LABORATOIRES SÉROBIOLOGIQUES
Member of cognis

Headquarters : Laboratoires Sérobiologiques S.A. • 3, rue de Seichamps - BP40 • F-54425 Pulnoy (France) • Fax. 03.83.29.98.65 • Tel. 03.83.29.97.91