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Influencing Dendritic Cells for an Increased Tolerance Threshold of Sensitive Skin
Introduction

The number of consumers who feel that their skin is especially sensitive has steadily increased over the past 20 years. Products for soothing sensitive skin usually increase the moisture content, strengthen the barrier function, or mitigate inflammatory processes. In many cases, however, the skin is neither dry nor particularly permeable or irritated, but is still perceived as sensitive.

The skin is constantly exposed to different environmental factors to which it usually responds adequately. For dangerous influences, dendritic cells alert other immune cells, thereby triggering a specific response. However, in the increasingly sterile human environment, early-state dendritic cells are often exposed to few foreign substances and are therefore unable to properly detect the nature and significance of a danger under certain circumstances, triggering inappropriate responses. Psychological stress also reduces the tolerance threshold of dendritic cells.

Overreactions can be mitigated by increasing the activation threshold of dendritic cells. The surface protein CD86 is a specific marker for the activity of dendritic cells. The expression of CD86 increases sharply in the presence of pathogenic antigens and pro-inflammatory cytokines. An active substance based on an extract from the leaves of Cestrum latifolium reduces the expression of CD86, thus reducing the activity of dendritic cells and lessening excessive defense responses. Sensitive skin is soothed, but its ability to simultaneously respond to environmental aggressors remains.

Characterization of Sensitive Skin

An individual predisposition to sensitive skin may have genetic, psychological or psychosomatic causes. Sensitive skin responds to exogenous, i.e., external triggers. These include inadequate skincare products or different environmental influences such as UV radiation or dry heated air.

In 2003, Yokota et al. published the recommendation to divide sensitive skin into three classes (1). Skin type I is characterized by a reduced barrier function with increased TEWL (transdermal water loss) and excessive flaking caused by a thinner and more permeable stratum corneum (2). Type II exhibits inflammatory changes, but the barrier function is intact. In skin type III, the barrier function is normal and there is no inflammation. This category best describes the state that consumers refer to as »sensitive skin«. From a physiological standpoint, one could describe skin that subjectively overreacts to environmental factors (3), or one could describe dis-
comfort without visible histological or clinical lesions (4).
In 2004, English described »subjectively irritated« skin as a mild manifestation of allergic contact dermatitis, which can be treated with corticosteroids (5). Their immunosuppressive effect is an indication that the immune system plays a role in so-called sensitive skin.

### The Immune System and Modern Ways of Life in Industrialized Countries

Atopic skin responses increase with increasing hygiene measures. Exposure to pathogens in the womb and the first years of life are the basis for the development of a functioning immune system. It learns to distinguish pathogenic from non-pathogenic factors. The frivolous use of antibiotics, the daily use of surfactant cleaning products, and the urban lifestyle, which reduces contact with animals, dramatically reduce exposure to foreign antigens. As a result, the skin increasingly responds inappropriately to influences that are quite harmless (6).

The stresses in the modern social and working environment are constantly increasing. It is known that stress affects the immune system and can even cause inflammatory responses. Psychological stress is an endogenous factor that has been shown to cause dendritic cells to mature and that contributes to the occurrence of contact allergies (7, 8, 9).

Recently, it was proven that social stress leads to activation of epidermal dendritic cells (10). An immune system that is on alert due to stress may potentially respond more readily, non-specifically, and inadequately to exogenous factors.

### Dendritic Cells in the Skin

Along with the respiratory tract and digestive tract, the skin is the region where people are regularly exposed to foreign bodies. Therefore, an effective protection system has developed in the course of evolution. First, the stratum corneum forms a mechanical barrier. Substances or microorganisms that can overcome this boundary can be destroyed in a non-specific response by substances released by keratinocytes such as antimicrobial peptides (defensins). Inflammation-promoting cytokines, such as TNF-α, interleukin-1 or interleukin-6 alert dendritic cells, are responsible for a specific immune response.

The importance of dendritic cells in the skin was not recognized for a long time. They were discovered in 1868 by the German pathologist Paul Langerhans, who could make the unusual cells visible under the microscope by marking them with gold chloride. However, he incorrectly assumed that they were nerve cells of the skin (11). It was not until 100 years later that Michel Prunières correctly described the «Langerhans cells»; namely, as cells that are able to trap foreign matter and elicit an immune response (12). These properties are common to all of the body’s immunocompetent cells.

Two cell types can be distinguished in the skin. The Langerhans cells are localized in the epidermis, in contrast to the dendritic cells of the dermis. In total, there are about 10^6 Langerhans cells in the entire human epidermis. Dendritic cells that are still inactive form a typical star-like shape. The cytoplasmic extensions (dendrites), which are over 10 µm long, radiate in all spatial directions starting from the cell body. These spurs are in constant motion; they move around and are withdrawn and then extended to another location. By behaving in this way, the dendritic cells can intercept invading pathogens and antigens in an optimal manner. Activation can be caused by contact with the pathogen, UV radiation, pro-inflammatory cytokines, or – as mentioned above – by stress, whereas differentiation from mature dendritic cells only occurs after contact with the antigen. The antigen is absorbed by phagocytosis. Guided by chemokines, the cells leave the peripheral tissue toward the next lymph node downstream in the lymphatic system. During the migration, the proteins of the foreign body are broken down into small peptide fragments, which can then be presented and recognized by the specific T lymphocytes with the involvement of the major histocompatibility complex (MHC class II complex). Due to the simultaneous distribution of certain cytokines, the dendritic cells activate the lymphocytes and ultimately trigger a specific cellular immune response.

During the migration to the lymph nodes, the morphology of the cells changes: The dendrites regress; membrane folds are formed for this. Cytokines, MHC II protein, and CD86 (»cluster of differentiation«) are expressed more intensively. Detection of the CD86 surface protein is very useful for determining the activity of the Langerhans or dendritic cells (13, 14).

### Surface Protein CD86

A reduction of the expression of the CD86 surface protein is indicative of a lesser degree of activation of dendritic cells. In this way, an excessive immune or inflammatory response is lessened. In this context, it is significant that the expression of CD86 is highly sensitive to sodium dodecyl sulfate (SDS) (15, 16, 17). In toxicological alternative tests to avoid animal experiments, SDS is widely used as a standard irritating substance, which has to be distinguished from a sensitizing substance, i.e., an allergen. Through frequent contact with surfactants, the Langerhans cells are in a constant state of alarm. Thus, the probability of an excessive specific response increases upon contact with less hazardous pathogens.

The influence of more than 100 different extracts and substances on the expression of the CD86 surface protein has been studied to identify a potential skin-soothing active ingredient. It was crucial for the effect to be mild, with no sustained impairment of the cells and with unconditional reversibility. This ensured that the activity potential and the ability to stimulate other parts of the immune system remain intact in the event of a serious threat.

### Efficacy Studies In Vitro

For determining the expression of CD86, dendritic cells were derived from CD14+ monocytes from blood in a patented in vitro model (18). In this way, it was possible to isolate Langerhans cells that express the CD207/ Langerin marker that is characteristic of this cell type, as well as dermal
dendritic cells that express the characteristic CD2097/DC-SIGN marker. The CD86 expression was determined by flow cytometry.

1. Preliminary Study
First, the expression of CD86 in a cohort of 30 healthy donors was determined. It was found that the expression has a variation of +/- 20% depending on the donor. This led to the basic hypothesis that an active ingredient that dampens the activity of dendritic cells by up to 20% does not affect their ability to function. This parameter was used as a criterion for the selection of an active ingredient. An extract from *Cestrum latifolium* met the requirements the best.

2. Effect of *Cestrum latifolium* on Expression of CD86
After 24 hours incubation in the presence of the immunosuppressant dexamethasone, which was used as a standard, the CD86 expression was reduced by 70%. The extract from *Cestrum latifolium*, in concentrations of between 0.05 and 1%, depending on the dose, lowered the expression of CD86. At a dose of 0.2%, the value fell by 18% (Fig. 1). This concentration was used for further investigations based on the initial hypothesis described above.

3. Reversibility of the Effect of *Cestrum latifolium* on Expression of CD86
This study examined whether the dendritic cells were able to completely recover their activity after treatment with the extract of *Cestrum latifolium*. For this purpose, the cells were first incubated for 24 hours in the presence of the active ingredient, and then the culture medium was replaced for 48 hours by one that did not contain the active ingredient. In the case of pretreatment with dexamethasone, the expression of CD86 did not reach the initial value and remained 27% below it. In the case of the extract from *Cestrum latifolium*, the previous expression was fully achieved once again after 48 hours (Fig. 2). The loss of activity is therefore reversible.

4. Influence of the Effect of *Cestrum Latifolium* on the Reactivity of the Langerhans Cells to Pathogens
After 24 hours of incubation with dexamethasone or the extract from *Cestrum latifolium*, a mixture of lipopolysaccharides and TNF-α was added to the culture medium. These substances simulate a bacterial infection; lipopolysaccharides are a typical component of the cell walls of bacteria,
and TNF-α is an inflammation-promoting enzyme. The cells pretreated with dexamethasone lost some of their ability to respond; the expression of CD86 reached only 47% of the untreated control cells. Pre-treatment with the extract from Cestrum latifolium had no effect: the expression of CD86 reached a value not significantly different from the control cells. Dendritic cells treated with the extract from Cestrum latifolium therefore respond without limitation to an attack from the outside (Fig. 3).

5. Influence of the Effect of Cestrum latifolium on the Ability of Dendritic Cells to Activate Other Immune Cells

The dendritic cells that were activated by LPS/TNF-α in a manner similar to the previously described experiment were incubated for 48 hours with T lymphocytes. The cells that were pretreated with the extract from Cestrum latifolium stimulated the proliferation of T lymphocytes to the same extent as in untreated control cells. In contrast, the stimulation rate in the dexamethasone pretreatment scenario only reached a fraction (Fig. 4). The extract from Cestrum latifolium therefore does not limit the allostimulatory properties.

6. Influence of Cestrum latifolium on the Motility of Dendritic Cells

Using video microscopy, this study investigated the extent to which the ability of dendritic cells to migrate is maintained. The bi-directional migration of the cells was recorded within 3 hours after a 24-hour incubation with active ingredient, then followed by a 48-hour regeneration phase, and finally after activation by LPS/TNF-α.

Figs. 5 to 10 show the significant difference in the migration rate when the cells were activated by the LPS/TNF-α mixture. Another important finding is that the sole addition of Cestrum latifolium extract does not influence the migration.

7. Influence of Cestrum latifolium on the Chemotactic Migration of Dendritic Cells

Dendritic cells migrate in the direction of certain cytokines in their environment, so-called chemokines. The chemokine CCL19 was selected for the study (19). The chemotactic migration of dendritic cells within three hours from a Transwell™ assay through a polycarbonate filter was determined after addition of the active ingredient (Fig. 11), after subsequent regeneration.
Fig. 7 Bi-directional migration of dendritic cells after treatment with *Cestrum latifolium* extract and subsequent regeneration phase. Video microscopy; the colored lines correspond to the distance covered in three hours.

Fig. 8 Bi-directional migration of dendritic cells after treatment with *Cestrum latifolium* extract and subsequent regeneration phase.

Fig. 9 Bi-directional migration of dendritic cells after treatment with *Cestrum latifolium* extract, subsequent regeneration phase, and stimulation with LPS/TNF-α. Video microscopy; the colored lines correspond to the distance covered in three hours.

Fig. 10 Bi-directional migration of dendritic cells after treatment with *Cestrum latifolium* extract and stimulation with LPS/TNF-α.

Fig. 11 Percentage of migrating cells after addition of *Cestrum latifolium* extract and regeneration phase.

Fig. 12 Percentage of migrating cells after addition of *Cestrum latifolium* extract or LPS/TNF-α.
There were no significant differences in the chemotactic behavior of dendritic cells found in any study. The *Cestrum latifolium* extract has no effect on the response of dendritic cells to chemokines.

8. Clinical Trial to Compare the Effect of the *Cestrum latifolium* Extract with a Skin-Soothing Product on the Market

31 subjects aged 20 to 69 were selected according to defined criteria for the characterization of sensitive skin. A 10% lactic acid solution was applied to the nasolabial fold area, and the intensity of tingling or burning was rated by the subjects at four fixed points in time using a scale from 0 to 9. Only subjects for whom the value was the same at least once or was above 3 were considered for the test panel.

A skin-soothing, redness-reducing commercial product from the upper price segment was used as a reference product in the clinical study. The product galenics were adjusted with *Cestrum latifolium* extract as the sole active ingredient. The subjects were not allowed to use any skin-soothing cosmetic product for a one week preparation phase. The reference product was then applied once daily for 3 weeks to one side of the face and the *Cestrum latifolium* formulation to the other side in a randomized manner.

For statistical analysis, the lactic acid stinging test that was already used for the selection of subjects was repeated after the period of application. In addition, the subjects subjectively rated five individual parameters (tingling, tightness, itching, warmth, burning) on a scale of 0 to 9. Furthermore, a dermatologist rated the »uniform complexion« and »red spots« criteria on a scale of 0 to 9 at the beginning and end of the study. Finally, the efficacy of the products was checked in an interview sheet (»strongly agree«, »agree«, »disagree«, »strongly disagree«). The positive and negative responses were totaled.

In the stinging test, the product containing the *Cestrum latifolium* formulation performed 30% better than the market product. Irritability decreased significantly by 20% compared to the initial value (Fig. 14). The result is also reflected in the evaluation by the subjects. There was a significant improvement in skin irritability attributed to both test products, whereby the *Cestrum latifolium* formulation was 7% better than the market reference (Fig. 15). For the *Cestrum latifolium* formulation, the dermatological evaluation also revealed a significant improvement in the homogeneity of the complexion, even against the market product, by 6%; the redness decreased by 19% (Fig. 16). These corrections in the skin’s appearance are illustrated in Fig. 17 based on the example of a test subject.
The results of the questionnaires indicate that the effects are perceptible. 90% of the subjects found that the Cestrum latifolium formulation contributed to a decreased irritability of the skin. 97% reported the skin feeling better or confirmed a skin-soothing effect. 71% saw a more brilliant complexion; 87% felt their skin to be better prepared for aggressive external influences. The results were improved to a statistically significant extent for all criteria and exceeded the market product (Fig. 18).

Summary

Excessive skin responses can be mitigated by increasing the threshold for activation of dendritic cells, whose surface protein CD86 is a specific marker for the activity of dendritic cells. The expression of CD86 increases sharply in the presence of pathogenic antigens and pro-inflammatory cytokines. An active substance based on an extract from the leaves of Cestrum latifolium reduces the expression of CD86, thus reducing the activity of dendritic cells and lessening excessive defense responses. Sensitive skin is soothed, but its ability to simultaneously respond to environmental aggressors remains.

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