

House Dust and Storage Mite Extracts Influence Skin Keratinocyte and Fibroblast Function

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Key Words

Cytokine · Chemokine · Mite · Atopic dermatitis · Allergy · Allergen · Macrophage colony-stimulating factor · Monocyte chemoattractant protein 1 · Growth-related oncogene α · Transforming growth factor α

Abstract

Background: The bodies of allergy-causing dust and storage mites likely contain many bioreactive molecules, including some that are allergenic. These molecules may penetrate the epidermis and dermis of the skin. However, little is known about the effects that most of the molecules from mites have on the function of cells in the skin, the overall inflammatory and immune reactions and the manifestation of allergic disease. The purpose of this research was to determine the response of cultured skin cells (keratinocytes and fibroblasts) to extracts of house dust and storage mites. **Methods:** Normal human epidermal keratinocytes and dermal fibroblasts were cultured with varying doses of extracts of the storage mites *Acarus siro*, *Chortoglyphus arcuatus* or *Lepidoglyphus destructor* or of the house dust mites *Dermatophagoides farinae*, *D. pteronyssinus* or *Euroglyphus maynei* in the absence or presence of lipopolysaccharide. Culture supernatants were collected 24 h later and assayed for the presence of various chemokines and cytokines. **Results:** Keratinocytes constitutively secreted interleukin (IL)-1 receptor antagonist/IL-1F3, growth-related oncogene α and transforming growth factor α , and these secretions were modulated

by extracts of 1 or more of the mites tested. Mite extracts also modulated the production of IL-6, IL-8, monocyte chemoattractant protein 1, macrophage colony-stimulating factor and vascular endothelial growth factor from fibroblasts.

Conclusions: The effects that mite extracts exerted on both keratinocytes and fibroblasts varied among the house dust mite species, among the storage mite species and between the house dust and storage mites. This study showed that extracts of mites contain substances that modulate the production of proinflammatory cytokines and chemokines secreted by normal human epidermal keratinocytes and dermal fibroblasts, and therefore may influence the course of pathophysiology in the skin in atopic dermatitis.

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Introduction

The bodies of allergy-causing dust and storage mites likely contain many bioreactive molecules, including some that are allergenic. One would expect that all of these mite-derived molecules in the home or work environment could contact and penetrate the skin or mucous membranes of the nose or eyes, or be inhaled into the bronchial airways. Mite extracts made from whole mite bodies that are used for skin testing and immunotherapy likely contain allergenic as well as nonallergenic bioactive molecules.

It has been shown that substances in extracts of house dust mites influence the function of lung epithelial cells, eosinophils and fibroblasts [1]. However, little is known about the effects that house dust and storage mite allergens and other molecules from these mites have on the function of cells in the skin, the overall inflammatory and immune reactions and the manifestation of allergic disease (atopic dermatitis).

House dust mite whole-body extracts contain many enzymes [2, 3]. Cork et al. [4] suggest that enzymes from dust mites may enhance the penetration of molecules (allergens) through the skin by modification of the desmosomes that seal the cells of the epidermis together. Patch tests in patients with atopic dermatitis have shown that Der p 1 and Der p 2 have both allergenic and proteolytic irritancy [5]. Components in house dust mite extracts are known to disrupt the integrity of lung epithelial cells [1, 6, 7]. Enzyme allergens (e.g. Der p 1, Der p 3, Der p 6, and Der p 9) and other components in house dust mite extracts are known to induce increased permeability of the lung epithelium, cleave tight-junction adhesion proteins between epithelial cells and induce apoptosis in cultured cell lines of human bronchial cells [6–10], cleave CD23 on activated B cells and CD25 from T cells [11, 12], and cause epithelial cells to detach [13]. Der p 1, Der p 3, Der p 9 or whole mite extract from *D. pteronyssinus* stimulate respiratory epithelial cells to produce multiple cytokines, including interleukin (IL)-6, IL-8, granulocyte/macrophage colony-stimulating factor (GM-CSF) and eotaxin (CCL11), and lung epithelial cells to produce prostaglandin E₂ [8, 14–17]. Der f 1 induces eosinophils to degranulate [18].

Some house dust and storage mite allergens are serine (Groups 3, 6, and 9) and cysteine (Group 1) proteases that may be acting through protease-activated receptors (PARs), a group of transmembrane proteins that occur on many types of cell. Four different PARs have been identified [1]. Cells with PARs include endothelial cells of blood vessels, fibroblasts, epithelium, mast cells and many airway cells, including neutrophils, monocytes, macrophages and smooth-muscle cells [1]. Many of these cells are involved in atopy, rhinitis and asthma. Stimulation of PARs on endothelial cells induces the expression of E- and P-selectin, intercellular-adhesion molecule 1, vascular-cell-adhesion molecule 1, and the production of the chemokines IL-8 and monocyte chemoattractant protein 1 (MCP-1) [1, 19–21]. Activation of PARs can increase eosinophil infiltration in chronic dermatitis. Stimulation of PAR-1 and -2 on dermal fibroblasts results in cell proliferation, production of platelet-derived growth factor,

vascular endothelial growth factor (VEGF), IL-8, MCP-1 and collagen [1].

Extracts of house dust mites may also contain endotoxins [22], and these substances may influence the function of many cell types via the toll-like receptors (TLRs) on the cells. Other bioactive components of mite bodies and feces have not been characterized, and these could elicit their effects via pathways other than through PARs and TLRs.

Physical or enzymatic disturbance of the epidermal stratum corneum might allow mite molecules to penetrate the stratum granulosum, stratum spinosum and stratum basale of the living epidermis, so that these substances enter the dermis. Evidence of this is that exposure to house dust mites and other environmental allergens has been associated with atopic dermatitis, and house dust mite avoidance measures have been shown to relieve the clinical symptoms in children with atopic dermatitis [4, 23, 24]. Mite allergens have been detected on the surface of the skin, and this mite material could penetrate the stratum corneum and contact or stimulate keratinocytes in the epidermis or fibroblasts in the dermis [25]. Furthermore, patch testing with house dust mite extracts has induced positive reactions in the skin of patients with atopic eczema [26]. Both keratinocytes and fibroblasts release cytokines and chemokines in response to physical and chemical stimulation, and these bioactive molecules affect vascular endothelial cell function and thus induce cutaneous inflammatory and immune reactions [27]. The effect that house dust and storage mite components have on keratinocytes and fibroblasts is unknown, but important in the manifestation of atopic dermatitis and other allergic diseases. The purpose of this research was to determine the response of cultured skin cells (keratinocytes and fibroblasts) to extracts of house dust and stored product mites.

Materials and Methods

Stored Product and House Dust Mite Extracts

Mite material was harvested and extracts prepared as previously described [28]. Briefly, aqueous whole-body extracts of the stored product mites *Acarus siro*, *Chortoglyphus arcuatus* and *Lepidoglyphus destructor* and of the house dust mites *Dermaphagoides farinae*, *D. pteronyssinus* and *Euroglyphus maynei* were prepared by extraction into glass-distilled water for 48 h at 4°C. The suspensions were then ground in a Ten Broeck homogenizer and centrifuged to remove insoluble material. The supernatants were collected, sterile-filtered (0.22 µm) into sterile vials and stored at 4°C. The Bradford Protein Assay [29] with bovine serum albumin as standard was used to determine the total protein concentration of each extract.

Normal Human Epidermal Keratinocytes: Adult

Pre-plated (48-well) adult normal human epidermal keratinocytes were purchased from Cambrex Bio Science Walkersville Inc. (Walkersville, Md., USA). Keratinocytes were maintained at 37°C in 6% CO₂ as recommended by supplier specifications in Keratinocyte Growth Medium 2 (KGM-2) supplemented with bovine pituitary extract, human epidermal growth factor, insulin, hydrocortisone, transferrin, epinephrine, gentamicine sulfate and amphotericin B. Plates remained at 37°C in 6% CO₂ until confluent, when they were used in cytokine detection studies as described below.

Normal Human Dermal Fibroblasts: Adult

Adult normal human epidermal fibroblasts were also purchased from Cambrex Bio Science. Fibroblasts were maintained at 37°C in 6% CO₂ as recommended by supplier specifications in Fibroblast Growth Medium 2 (FGM-2) supplemented with fetal bovine serum, fibroblastic growth factor, insulin, gentamicine sulfate and amphotericin B. Cells were maintained in BD Falcon (Bedford, Mass., USA) 75 cm² tissue-culture-treated flasks until confluent, at which time cells were plated into Costar 96-well tissue-culture-treated plates (Corning Inc., Corning, N.Y., USA) and allowed to again reach confluence before being used in cytokine detection studies. All studies were performed on cells between passages 4 and 19.

Challenge of Human Skin Cells with Mite Extracts

Time course studies were performed to determine the optimal time for analysis of cytokine expression. A previous study had determined the optimal concentration of *Escherichia coli* lipopolysaccharide (LPS; Sigma Chemical Co., St. Louis, Mo., USA) [30]. Experiments were initiated when the cultures in the plates reached confluence. Medium was aspirated from the plates and replaced with the appropriate medium (KGM-2 for keratinocytes or FGM-2 for fibroblasts) alone or containing 12.5, 25.0, 50.0 or 100 µg/ml of the various mite extracts in the absence or presence of 50 ng/ml LPS. For each experiment (plate of cells) the stimulation was performed in triplicate wells. Plates were maintained at 37°C in 6% CO₂ for 24 h. Supernatants were then collected and stored at -80°C for later analysis of cytokine expression.

Cytokine Detection

Cytokine secretion into the culture supernatant was measured using cytokine-specific enzyme-linked immunosorbent assays (ELISA). DuoSet® ELISA Development System kits for human IL-1 receptor antagonist/IL-1F3 (IL-1ra), IL-1α (IL-F1), IL-3, IL-7, IL-10, IL-12 p70, IL-15, eotaxin (CCL11), granulocyte colony stimulating factor (G-CSF), GM-CSF, growth-related oncogene α/CXCL1 (GRO-α), macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein 1 α/CCL3 (MIP-1α), stem cell factor receptor (SCF R/c), transforming growth factor α (TGF-α), tumor necrosis factor α/TNFSF1A (TNF-α), and VEGF were obtained from R&D Systems Inc. (Minneapolis, Minn., USA). IL-8 and IL-1β screening sets were obtained from Endogen (Pierce Biotechnology Inc., Rockford, Ill., USA). MCP-1 (CCL2) and IL-6 ELISA kits were obtained from eBioscience (San Diego, Calif., USA). All assays were performed according to manufacturers' instructions.

All cell challenge experiments were performed several times. The data presented here are averages ± standard errors of the mean (SEM) for triplicate wells from representative experiments.

Results

Modulation of Cytokine Secretion from Keratinocytes

Extracts of the stored product mites *A. siro* and *C. arcuatus* and the house dust mites *D. farinae*, *D. pteronyssinus* and *E. maynei* did not stimulate secretion of IL-1β, IL-3, IL-6, IL-7, IL-10, IL-12, IL-15, GM-CSF or MCP-1 (CCL2) by keratinocytes.

Interleukin 1 Receptor Antagonist

Keratinocytes constitutively secreted IL-1ra and this secretion was not appreciably influenced by stimulation with LPS (fig. 1, 2). *D. farinae* and *D. pteronyssinus* did not influence the production of IL-1ra by keratinocytes, while *E. maynei* at low doses reduced constitutive secretion. In contrast, the storage mite *A. siro* at 100 µg/ml and all doses of *C. arcuatus* reduced the constitutive secretion of IL-1ra.

Growth Related Oncogene α (CXCL1)

Cultured keratinocytes also constitutively secreted GRO-α (fig. 1, 2). Stimulation with *A. siro* extract reduced constitutive secretion at high doses (50 and 100 µg/ml), and *C. arcuatus* inhibited constitutive secretion at all doses. Low doses of *D. farinae*, *D. pteronyssinus* and *E. maynei* induced keratinocytes to produce amounts of GRO-α that were above constitutive levels. There was no effect of LPS on either constitutive or mite-modulated GRO-α production.

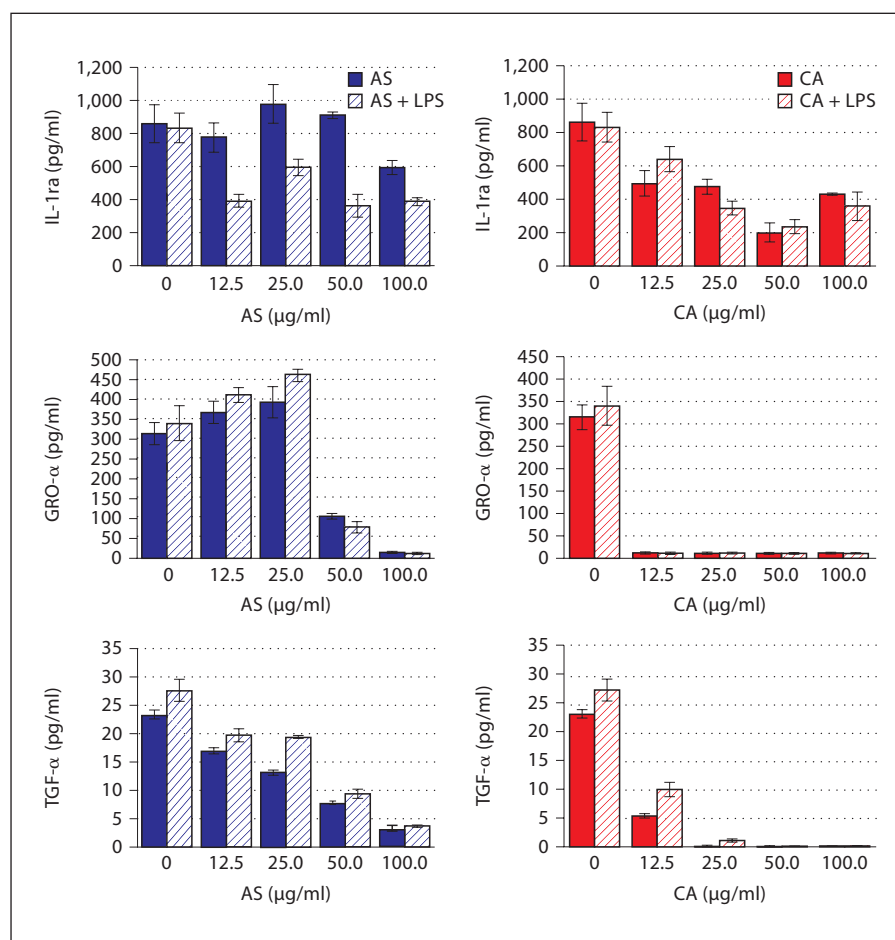
Transforming Growth Factor α

The storage mites *C. arcuatus* and *A. siro* and low doses of the house dust mite *E. maynei* reduced constitutive secretion of the proinflammatory cytokine TGF-α, while *D. farinae* and *D. pteronyssinus* had no effect on its production (fig. 1, 2). TGF-α production in the absence or presence of any mite extract was unaffected by the addition of LPS.

Modulation of Cytokine Secretion from Fibroblasts

Human dermal fibroblasts did not secrete IL-1ra, IL-1α, IL-1β, IL-7, IL-15, G-CSF, GM-CSF, MIP-1α (CCL3), SCF R/c, TNF-α or eotaxin (CCL11) either constitutively or when stimulated with extracts of the stored prod-

Fig. 1. Modulatory effect of varying doses of storage mite extracts on the secretion of IL-1ra, GRO- α and TGF- α by normal human epidermal keratinocytes in the absence or presence of LPS. AS = *Acarus siro*; CA = *Chortoglyphus arcuatus*. Data are means $\pm$ SEM (n = 3).



uct mites *A. siro*, *C. arcuatus* or *L. destructor* or of the house dust mites *D. farinae*, *D. pteronyssinus* or *E. maynei*.

Interleukin 6

Extracts of *A. siro*, *C. arcuatus*, *L. destructor* and *D. farinae* induced dose-dependent increases in secretion of IL-6 by fibroblasts, and this was enhanced in the presence of LPS (fig. 3, 4). *D. pteronyssinus* and *E. maynei* did not induce IL-6 secretion, and in fact these extracts down-regulated LPS-induced IL-6 secretion.

Interleukin 8 (CXCL8)

Fibroblasts constitutively produced large amounts of IL-8, and this production was increased by stimulating the cells with *D. farinae*, *D. pteronyssinus* or *E. maynei* at all doses (fig. 3, 4). Extracts of all 3 storage mite species tested also induced a dose-dependent secretion of IL-8. LPS induced enhanced IL-8 secretion that was not

modulated by coinubation with any of the mite extracts.

Monocyte Chemoattractant Protein 1

C. arcuatus, *L. destructor* and *D. farinae* induced secretion of MCP-1, but *A. siro*, *D. pteronyssinus* and *E. maynei* did not (fig. 3, 4). *A. siro*, *L. destructor*, *D. farinae*, *D. pteronyssinus* and *E. maynei* all inhibited the LPS-induced secretion of MCP-1, an effect that was not observed in the presence of *C. arcuatus*.

Macrophage Colony-Stimulating Factor

Fibroblasts constitutively secreted M-CSF (fig. 3, 4). Low doses of the storage mites *A. siro*, *C. arcuatus* and *L. destructor* inhibited secretion of M-CSF, while the dust mites *D. farinae* and *D. pteronyssinus* stimulated the secretion of this proinflammatory cytokine. Neither constitutive nor mite-modulated secretion was affected when cells were coinubated with LPS.

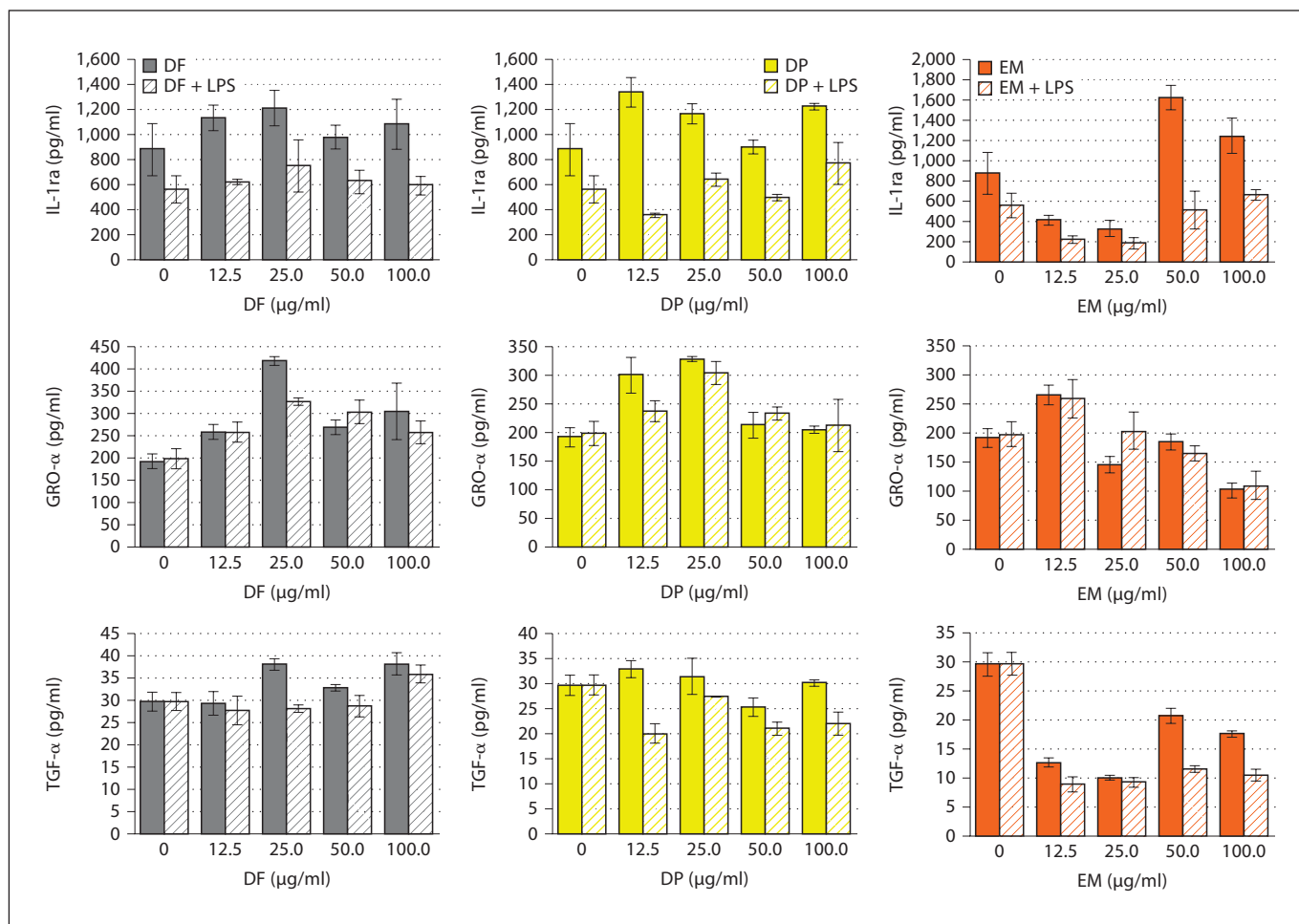


Fig. 2. Modulatory effect of varying doses of house dust mite extracts on the secretion of IL-1ra, GRO-α and TGF-α by normal human epidermal keratinocytes in the absence or presence of LPS. DF = *Dermatophagoides farinae*; DP = *D. pteronyssinus*; EM = *Euroglyphus maynei*. Data are means $\pm$ SEM (n = 3).

Vascular Endothelial Growth Factor

Stimulation of fibroblasts with *A. siro*, *C. arcuatus* or *L. destructor* resulted in a dose-dependent increase in production of VEGF (fig. 3). VEGF production was not appreciably altered by incubation of the cells with LPS. None of the house dust mite extracts induced secretion of VEGF.

Discussion

The skin is exposed to house dust and storage mite antigenic and nonantigenic substances that may penetrate the epidermis and enter the dermis. As mite products enter the skin, epidermal keratinocytes and dermal fibro-

blasts, which are important for the structural integrity and function of the skin, may be contacted and stimulated. In vivo, mite products may trigger keratinocytes and fibroblasts to modify their production of cytokines and chemokines, which may directly influence the function of other cells in the vicinity, such as dermal microvascular endothelial cells, which control cellular extravasation and thus local inflammation and immune reactions. Keratinocytes and fibroblasts are capable of producing a wide array of cytokines and chemokines [27, 31]. In this study, we found that both normal human keratinocytes and fibroblasts cultured in the presence of dust or storage mite extracts modulated the secretion of specific cytokines and chemokines by (1) inducing the secretion of cytokines and chemokines that were not constitutively secreted, (2) in-

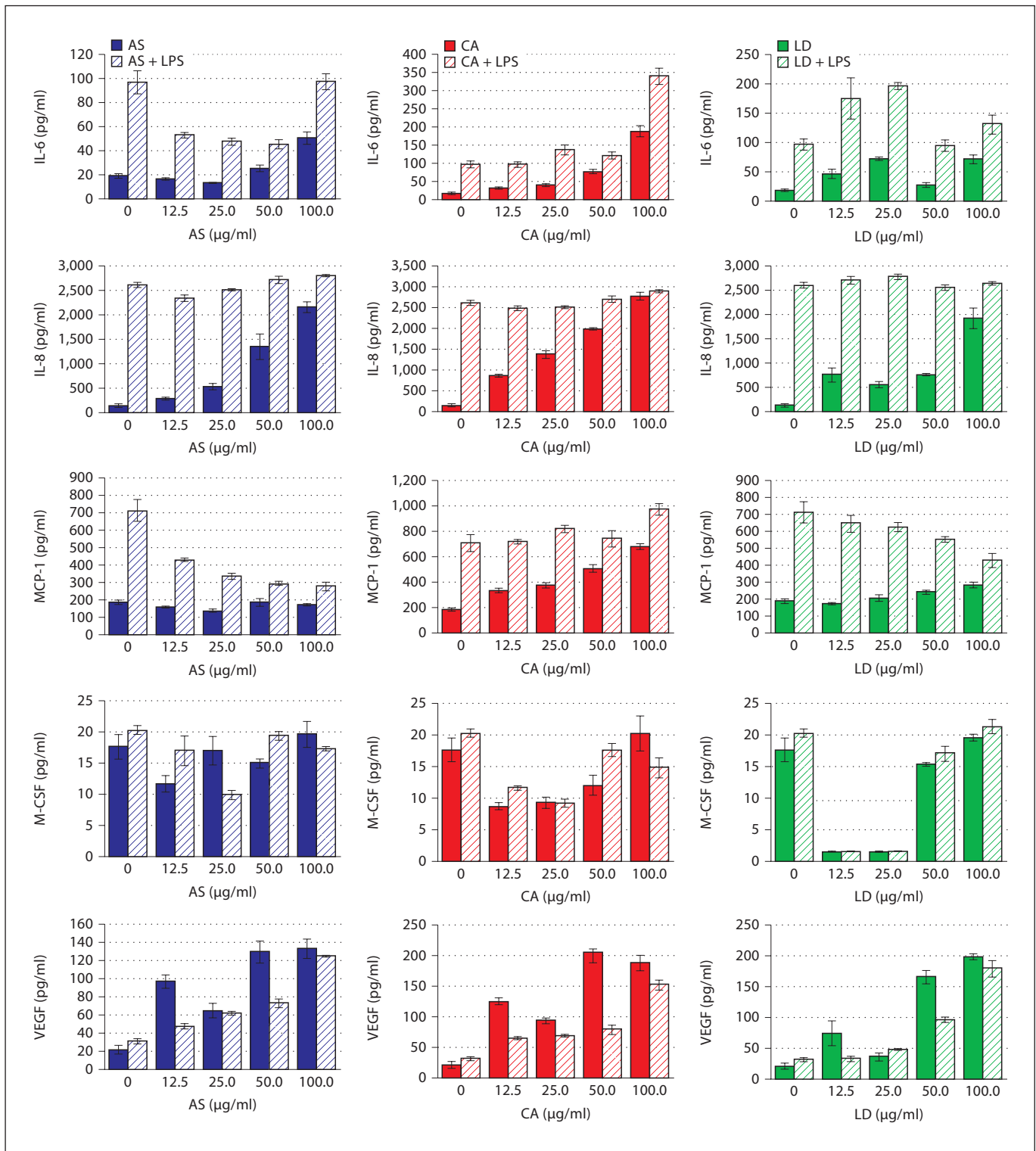


Fig. 3. Modulatory effect of varying doses of storage mite extracts on the secretion of IL-6, IL-8, MCP-1, M-CSF and VEGF by normal human dermal fibroblasts in the absence or presence of LPS. AS = *Acarus siro*; CA = *Chortoglyphus arcuatus*; LD = *Lepidoglyphus destructor*. Data are means $\pm$ SEM (n = 3).

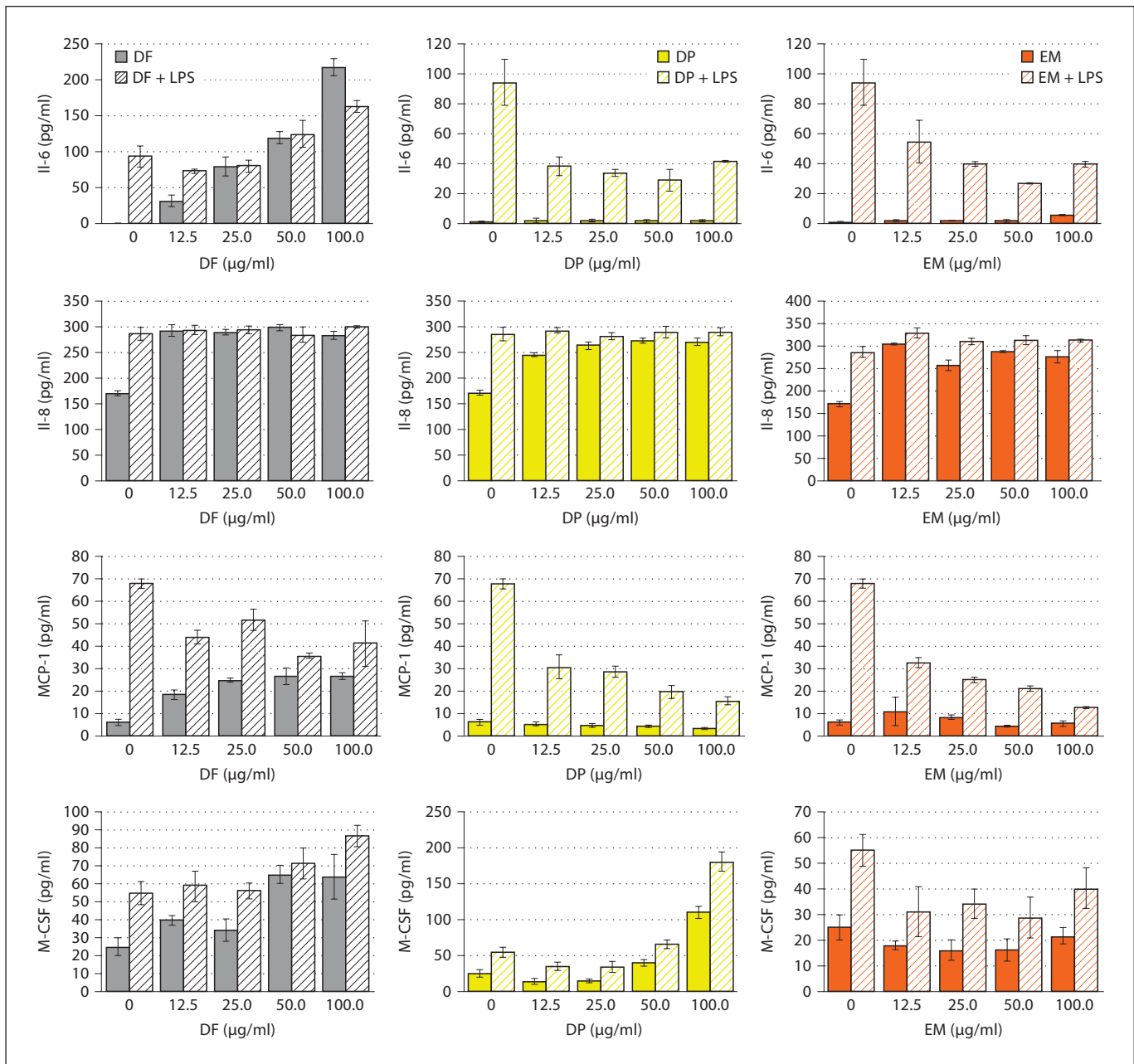


Fig. 4. Modulatory effect of varying doses of house dust mite extracts on the secretion of IL-6, IL-8, MCP-1, and M-CSF by normal human dermal fibroblasts in the absence or presence of LPS. DF = *Dermatophagoides farinae*; DP = *D. pteronyssinus*; EM = *Euroglyphus maynei*. Data are means $\pm$ SEM (n = 3).

creasing the production of cytokines and chemokines above the levels that were constitutively secreted or were induced by LPS, or (3) reducing the level of secretion of cytokines and chemokines that were constitutively secreted or that were induced by stimulation of the cells with LPS. The effects that mite extracts exerted on keratino-

cytes and fibroblasts varied among the house dust mite species and storage mite species and between the house dust and storage mites (table 1).

It is difficult to judge the *in vivo* effects of our findings. Chemicals, irritants and physical damage to the skin stimulate the production of proinflammatory cytokines,

Table 1. Summary of the modulatory effects of mite extracts on cytokine production by normal human epidermal keratinocytes and dermal fibroblasts

	Medium	LPS	AS		CA		LD		DF		DP		EM	
Normal Human Epidermal Keratinocytes														
IL-1ra	+	=	Lo ↔	Hi ↓	↓		ND		↔		↔		Lo ↓	Hi ↑
GRO-α	+	=	Hi ↓		↓		ND		Lo ↑	Hi ↔	Lo ↑	Hi ↔	Lo ↑	Hi ↔
TGF-α	+	=	↓		↓		ND		↔		↔		↓	
Normal Human Dermal Fibroblasts														
IL-6	+	↑	↑		↑		↑		↑			↔	LPS ↓	↔ ↓
IL-8	+	↑	↑		↑		↑		↑		↑	LPS =	↑	LPS =
MCP-1	+	↑	↔	LPS ↓	↑	LPS =	↑	LPS ↓	↑	LPS ↓	↔	LPS ↓	↔	LPS ↓
M-CSF	+	=	Lo ↓	LPS =	Lo ↓	LPS =	Lo ↓	LPS =	↑	LPS =	↑	LPS =	↔	
VEGF	+	↑	↑	LPS =	↑	LPS =	↑	LPS =	0		0		0	

AS = *Acarus siro*; CA = *Chortoglyphus arcuatus*; LD = *Lepidoglyphus destructor*; DF = *Dermatophagoides farinae*; DP = *D. pteronyssinus*; EM = *Euroglyphus maynei*; Lo = Low dose of mite extract; Hi = High dose of mite extract; ↑ = levels increased relative to media or LPS control (as indicated); ↓ = levels decreased relative to media or LPS control (as indicated); ↔ = levels unchanged relative to media or LPS control (as indicated); ‘=’ = levels in the presence of LPS equal to those in its absence; ND = not determined.

including IL-1α, IL-1β, IL-6, GRO-α, TNF-α, and GM-CSF [27, 31, 32]. Scratching of the skin itself by the patient induces fibroblasts and keratinocytes to produce and release proinflammatory cytokines and chemokines that in turn exacerbate inflammation (atopic dermatitis). It is significant that both house dust and storage mites influenced the secretion of GRO-α from keratinocytes. This chemokine is chemotactic for the extravasation of leukocytes from the blood into the dermis during inflammation. It is interesting that neither house dust nor storage mite extracts stimulated the secretion of the proinflammatory cytokines IL-1β or GM-CSF by either fibroblasts or keratinocytes or of IL-1α by fibroblasts. However, some doses of the house dust mite *E. maynei* and the storage mites *C. arcuatus* and *A. siro* reduced secretion of IL-1ra. Since IL-1ra is a competitive inhibitor of the proinflammatory cytokine IL-1 by blocking activation of the IL-1 receptor on effector cells, the net effect of its down-regulation by these particular mite species would be to

promote the inflammatory reaction to these mites if other cells in the vicinity (e.g. Langerhans cells, macrophages) were stimulated to produce IL-1. These results are consistent with previous reports that scabies mite extracts did not induce IL-1 secretion by cultured keratinocytes and fibroblasts [30]. However, when live *S. scabiei* mites were allowed to burrow into the epidermis of human skin equivalents (containing both keratinocytes and fibroblasts) IL-1α and IL-1β were released, presumably from keratinocytes [33]. Likewise, scabies extracts induced increases in the production of IL-6, IL-8, G-CSF and VEGF by fibroblasts [30]. In a study by Tomee et al. [8], *L. destructor* and *D. pteronyssinus* were found to induce significant secretion of IL-6, IL-8 and MCP-1 by lung alveolar type-II epithelial cells (A549). Also, in that study, *D. pteronyssinus* induced a significant increase in IL-8 production from nasal epithelial cells from nonatopic individuals, while a decrease in the production of IL-8 was found with higher doses of *L. destructor* [8].

In this study, we examined the influence of mite extracts on keratinocytes and fibroblasts separately; therefore, cellular interactions were not investigated. The use of monocultures of cells does have some limitations. In vivo, these cells are separated by the basement membrane, so they do not physically interact in the intact skin. Although cellular interactions were not investigated, it is likely that this occurs in vivo, and this interaction may influence the response of these cells to the mite substances. Cellular interactions are possible when enzymes from mites disrupt the basement membrane, or when cytokines from each cell type diffuse inward and outward between the epidermis and dermis. Studies showed that keratinocytes in human skin equivalents (containing both fibroblasts and keratinocytes) release IL-1 α and IL-1 β in response to scabies mites, while isolated cells did not produce these substances in response to scabies extract [33]. This suggests that the interaction of these cells is important, and that together they may behave differently than when separated. A study by Smola et al. [34] showed that the presence of keratinocytes significantly upregulated mRNA expression levels by fibroblasts. Likewise, Maas-Szabowski et al. [35] found that expression of mRNA by fibroblasts and keratinocytes for keratinocyte growth factor, IL-1 α and IL-1 β was greater when the cells were cultured together than when they were cultured alone. These interactions and reciprocal modulation in response to mites remain to be investigated.

The mechanism by which a mite extract can affect constitutive and LPS-induced cytokine secretion in opposite directions remains unclear. We observed this, and it suggests that induction of secretion involves a separate pathway or mechanism to inhibition, and is likely the result of different molecules in the same extract. Perhaps something in the mite extract blocks TLRs that are stimulated by LPS. The fact that different mite species elicited different responses, and in some cases downregulated LPS-induced secretion while inducing increased constitutive secretion, indicates that these mites are the sources of many different molecules that stimulate or inhibit cell function, apparently acting through different receptors such as TLRs, PARs or others. A substance in a mite extract could block a TLR, preventing it from being activated by its usual ligand. The substances in the extracts responsible for the modulation of keratinocyte and fibroblast function are not yet known. They may include proteases that act through PARs. Many of the mite allergens are proteases. Tomee et al. [8] have found that both *L. destructor* and *D. pteronyssinus* extracts stimulate production of IL-8 by cultured airway epithelial cells exposed to

mite extract. Enzymatic analysis of gel filtration fractions showed that the IL-8 activity was greatest in fractions containing the highest protease activity, which suggests that PARs were involved. Or, mite extracts may include endotoxins that act through TLRs by stimulating them. Extracts of *D. farinae* and *D. pteronyssinus* have both been shown to contain endotoxins [22]. Another possibility is that a mite extract could induce the production of a single cytokine, which then induces the subsequent production of other cytokines in an autocrine fashion.

Conclusion

This study showed that extracts of mites contain substances that modulate the production of proinflammatory cytokines and chemokines, secreted by normal human epidermal keratinocytes and dermal fibroblasts, which may contribute to the development of skin inflammation and immunologic reactions and to the pathophysiology in the skin in atopic dermatitis. Our data provide new insights and additional perspectives into the role (and mechanisms) of dust mites and storage mites in skin inflammation. Much remains to be understood regarding the role of these mites in the cellular interactions involved in atopic dermatitis and the specific molecules involved and the mechanisms for how these molecules modulate keratinocyte and fibroblast activity. All of these mite-modulated responses may also be modified by exogenous factors such as bacteria and their products, chemical irritants, washing and soaps and detergents [4].

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