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Response of Human Skin Equivalents to *Sarcoptes scabiei*

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Abstract

Studies have shown that molecules in an extract made from bodies of the ectoparasitic mite, *Sarcoptes scabiei* De Geer, modulate cytokine secretion from cultured human keratinocytes and fibroblasts. In vivo, in the parasitized skin, these cells interact with each other by contact and cytokine mediators and with the matrix in which they reside. Therefore, these cell types may function differently together than they do separately. In this study, we used a human skin equivalent (HSE) model to investigate the influence of cellular interactions between keratinocytes and fibroblasts when the cells were exposed to active/burrowing scabies mites, mite products, and mite extracts. The HSE consisted of an epidermis of stratified stratum corneum, living keratinocytes, and basal cells above a dermis of fibroblasts in a collagen matrix. HSEs were inoculated on the surface or in the culture medium, and their cytokine secretions on the skin surface and into the culture medium were determined by enzyme-linked immunosorbent assay. Active mites on the surface of the HSE induced secretion of cutaneous T cell-attracting chemokine, thymic stromal lymphopoietin, interleukin (IL)-1 α , IL-1 β , IL-1 receptor antagonist (IL-1ra), IL-6, IL-8, monocyte chemoattractant protein-1, granulocyte/macrophage colony-stimulating factor, and macrophage colony-stimulating factor. The main difference between HSEs and monocultured cells was that the HSEs produced the proinflammatory cytokines IL-1 α and IL-1 β and their competitive inhibitor IL-1ra, whereas very little of these mediators was previously found for cultured keratinocytes and fibroblasts. It is not clear how the balance between these cytokines influences the overall host response. However, IL-1ra may contribute to the depression of an early cutaneous inflammatory response to scabies in humans. These contrasting results illustrate that cell interactions are important in the host's response to burrowing scabies mites.

Keywords

scabies mites; human skin equivalents; interleukin-1 receptor antagonist; immuno-modulation; skin cell cytokine secretion

The ectoparasitic mite, *Sarcoptes scabiei* De Geer, is the source of molecules that modulate the function of many cell types involved in the host's innate and immune responses to them. These cells include human dermal keratinocytes and fibroblasts (Arlian et al. 2003, Mullins et al. 2009), peripheral blood mononuclear cells and dendritic cells derived from them (Arlian et al. 2004), T-regulatory lymphocytes (Arlian et al. 2006), and skin endothelial cells of the microvasculature (Elder et al. 2006, 2009). This adaptation may allow mites to circumvent the inflammatory and immune responses that eliminate them during an early infestation and favor the parasites becoming established in the host skin. Evasion of the specific components of the host's inflammatory and immune responses is a common strategy shared by many endo- and ectoparasites. For example, scabies mite-inactivated protease paralogs have been demonstrated to inhibit the human complement system (Bergstrom et al. 2009). Anti-inflammatory, anti-

immune, and anti-complement strategies have been identified in ticks and other invertebrate parasites (Ramachandra and Wikel 1992, Diaz et al. 1997, Wikel 1999, Maxwell et al. 2005, Nunn et al. 2005, Daix et al. 2007, Hepburn et al. 2007, Schroeder et al. 2007, Zipfel et al. 2007).

Previous in vitro studies showed that monocultures of normal human keratinocytes and fibroblasts stimulated with *S. scabiei* extracts modulated their secretion of specific cytokines and chemokines (Arlian et al. 2003, Mullins et al. 2009). In vivo, keratinocytes and fibroblasts interact with each other by contact and cytokine mediators and with the collagen matrix in which they reside, and therefore, these cells may not respond in the same way as they do in isolation (monoculture). Also, substances present in mite extracts may not accurately represent what these cells are exposed to in vivo during an active infestation with this parasite. Burrowing mites secrete salivary solution that aids in penetration of the epidermis (Arlian et al. 1984). The live mite salivary secretions may contain more, less, or different bioactive molecules than are present in extracts made from mite bodies. Physical stimulation of the skin such as scratching is also known to induce keratinocytes and fibroblasts to produce proinflammatory cytokines (Homey et al. 2006). Therefore, actively burrowing mites may physically stimulate epidermal keratinocytes and even fibroblasts in the dermis. We previously found that mites burrowing into human skin equivalents (HSE; keratinocytes grown over fibroblasts in a collagen matrix) produced interleukin (IL)-1 α and IL-1 β (Arlian et al. 1996b), but cultured cells alone did not produce appreciable amounts of these cytokines in response to stimulation with mite extract (Arlian et al. 2003).

The purpose of this research was to investigate the effect of living scabies mites and mite extracts on cytokine and chemokine secretion by keratinocytes and fibroblasts in a HSE model. The HSE model (Epiderm EFT-400) is structurally much like normal human skin in vivo and consists of an epidermis of stratified stratum corneum, living keratinocytes, and basal cells above a dermis of fibroblasts in a collagen matrix.

Materials and Methods

Scabies Mites and Extract

S. scabiei variety *canis* De Geer were collected by aspiration onto a 400-mesh (38- μ m) stainless steel screen after they had migrated from crusts collected from infested rabbits. For extract preparation, mites were killed by freezing and later were homogenized in endotoxin-free water, as previously described (Elder et al. 2009). The soluble material was collected after centrifugation, and the supernatant was sterile filtered into sterile vials. Protein concentrations were determined by the Bradford protein assay using bovine serum albumin as the standard (Bradford 1976). For inoculation onto HSEs, mites were collected onto the screen and washed by aspiration of sequential 4-ml volumes of phosphate-buffered saline with 0.05% Tween-20, endotoxin-free water, and 70% ethanol.

HSE Challenge

EpiDerm EFT-400 full-thickness HSEs and medium were purchased from MatTek (Ashland, MA). Upon arrival, HSEs on their supports were transferred to new 6-well culture plates containing 3.0 ml of fresh medium/well, and plates were placed in a 37°C incubator with 5–7% CO₂. The next day, HSEs were transferred to new plates containing 5.0 ml of fresh medium or of medium containing scabies extract at a final protein concentration of 100 μ g/ml ($n = 5$). Several hundred live mites were then transferred to the surface of one set ($n = 5$) of HSEs or into the lower well of another set ($n = 5$). Another set ($n = 5$) was inoculated on the surface with 100 μ g of scabies extract protein, whereas the final set ($n = 4$) remained as untreated controls. HSEs were then returned to the incubator.

At 6, 12, 18, and 24 h after inoculation, each HSE on its support was lifted from the well, the medium was mixed, and a 550- μ l aliquot was removed from the sample well and frozen at -80°C . At the conclusion of the experiment (48 h), all medium was collected from the well and the surface of each HSE was washed with 500 μ l of medium that was collected and also frozen at -80°C .

Cytokine Measurements

The concentrations of various cytokines in the culture medium aliquots and surface washes were measured using enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, MN). Data are from one representative experiment of several replicates and are presented as mean $\pm$ SEM. Statistical significance was determined using a single-factor analysis of variance analysis with differences from control with $P < 0.05$ judged as significant.

Results

Cutaneous T Cell-Attracting Chemokine (CTACK/CC Chemokine Ligand [CCL] 27)

Burrowing mites on the surface of the HSE induced significant secretion of CTACK onto the surface (stratum corneum) of the HSE (Table 1). Scabies extract on the surface of HSEs induced secretion of CTACK into the culture medium, whereas neither extract in the medium nor live mites on the HSE surface or in the culture medium increased CTACK secretion above constitutive levels (Fig. 1). Interestingly, mite extract added to the culture medium did induce increased CTACK secretion at 6 h compared with the control, but this significant difference disappeared by 18 h (Fig. 1).

Thymic Stromal Lymphopoietin (TSLP)

Live mites burrowing into the surface of the HSEs induced TSLP secretion onto the skin surface and into the culture medium (Table 1; Fig. 1). Scabies extract inoculated into the medium induced a substantial secretion of TSLP into the culture medium at 6 h, but the concentration waned with time (Fig. 1).

Growth-Related Oncogene α (GRO α /CXC Chemokine Ligand 1)

HSEs constitutively secreted GRO α (Table 1; Fig. 1). Mites inoculated onto the HSE surface induced the most GRO α secretion both onto the HSE surface (Table 1) and into the culture medium (Fig. 1) compared with the other stimuli. Scabies extract inoculated onto the HSE surface also induced a 1.6-fold increase in GRO α secretion onto the HSE surface (Table 1), but it down-regulated the constitutive secretion of GRO α into the well by $>50\%$ (Fig. 1).

Transforming Growth Factor α (TGF α)

There was no constitutive secretion of TGF α . None of the stimuli induced TGF α secretion onto the HSE surface (Table 1), but small amounts of TGF α were detected in the wells at 48 h in response to the burrowing of live mites into the HSE surface (data not shown).

Interleukin-1 α (IL-1 α), IL-1 β , and IL-1 Receptor Antagonist (IL-1ra)

Mites burrowing into the epidermis on the surface of HSEs induced secretion of significantly increased amounts of IL-1 α , IL-1 β , and IL-1ra onto the skin-equivalent surface (Table 1). The burrowing of mites into the HSE surface also induced secretion of significant amounts of IL-1 α into the culture medium (Fig. 1).

IL-6 and IL-8 (CXC Chemokine Ligand 8)

Live mites burrowing into the surface also induced the secretion of significantly increased levels of IL-6 and IL-8 onto the surface of HSEs (Table 1) and into the culture medium (Fig. 1). Live mites or scabies extract present in the culture medium in the wells below the HSEs also induced significantly increased secretion of IL-6 and IL-8 into the medium (Fig. 1). Scabies extract applied either onto the surface of the HSE or in the culture medium induced a 2- to 3-fold increase in IL-6 and IL-8 secretion onto the HSE surface (Table 1).

Monocyte Chemoattractant Protein-1 (MCP-1/CCL2)

This chemokine was constitutively produced by HSEs (Table 1; Fig. 1). Surface secretion of MCP-1 was significantly increased by burrowing live mites (Table 1), whereas no other treatment significantly altered MCP-1 secretion either onto the HSE surface (Table 1) or into the culture medium in the lower well (Fig. 1).

Granulocyte and Granulocyte/Macrophage Colony-Stimulating Factors (G-CSF and GM-CSF)

These growth factors were constitutively secreted by HSEs (Table 1; Fig. 1). By 48 h, the level in medium of mite-infested HSEs was significantly higher than all other levels that were essentially identical (Fig. 1). Mite-infested HSEs also secreted increased levels of GM-CSF and G-CSF onto their surfaces (Table 1).

Macrophage Colony-Stimulating Factor (M-CSF)

Mites burrowing into the HSE induced the secretion of large amounts of M-CSF onto the surface, and these levels far exceeded the amounts produced in response to any of the other test conditions (Table 1).

Vascular Endothelial Growth Factor (VEGF)

HSEs constitutively secreted VEGF. At 48 h, only those HSEs exposed to mites in the well secreted significantly more VEGF into the culture medium than the controls (Fig. 1), although most other stimuli also increased VEGF secretion on the surface (Table 1) and into the culture medium (Fig. 1).

IL-3 and Thymus- and Activation-Regulated Cytokine (TARC/CCL17)

In a preliminary experiment using a different set of HSEs, we assayed the medium for IL-3 and TARC secretion. Neither IL-3 nor TARC was detected at any time in response to any stimulus (Table 2).

Discussion

Chemical and physical cell-cell interactions in an extracellular matrix framework and between the cells and the matrix are important aspects that determine how cells respond to stimuli in vivo in the skin. Previous studies showed that monocultured keratinocytes and fibroblasts increase constitutive levels of secretion or decrease proinflammatory-induced secretion levels of specific cytokines and chemokines in response to unidentified molecules in *S. scabiei* extracts (Table 2). A limitation of the monoculture system is that there is an absence of direct communication between the keratinocytes and fibroblasts, and between these cells and the matrix, and with other cells that also may be present in the skin (e.g., Langerhans cells, other dendritic cells, leukocytes). Thus, one cell type cannot influence the behavior of another in response to scabies molecules because the cells lack intercellular interactions and associations with the collagen matrix of the normal skin. We have attempted to model the in vivo situation to some extent by costimulation of these cells with scabies extract and proinflammatory

cytokines from other cells that are most likely present in the scabietic lesion (Elder et al. 2009, Mullins et al. 2009).

In this study, we used HSEs to more fully investigate cellular interactions that occur between keratinocytes and fibroblasts when the cells are exposed to infesting mites, mite products, and mite extracts. Keratinocytes and fibroblasts are the two primary structural cells of the skin. One shortcoming of this model system is that the cytokine secretions measured represent the total contribution of the two interacting cells, and thus, the contribution of each cell type alone cannot be identified.

Our previous studies found that normal human keratinocytes up-regulated secretion of IL-6, VEGF, GRO α , TGF α , G-CSF, and CTACK, and down-regulated constitutive and IL-1 α , IL-1 β , tumor necrosis factor- α (TNF α) plus IL-17, and lipopolysaccharide-induced secretion of IL-8, and the constitutive secretion of IL-1ra when cultured in the presence of *S. scabiei* extract (Table 2; Arlian et al. 2003, Mullins et al. 2009). Scabies extract did not appreciably stimulate secretion of IL-1 α or IL-1 β by keratinocytes, but it did reduce their constitutive secretion of IL-1ra (Arlian et al. 2003). Likewise, normal human fibroblasts cultured in the presence of *S. scabiei* extract up-regulated secretion of IL-6, G-CSF, and VEGF, and down-regulated secretion of GM-CSF and IL-8 (after 8-h stimulation) that was induced by stimulation of the cells with IL-1 α , IL-1 β , and TNF α plus IL-17 (Mullins et al. 2009). In addition, IL-8 secretion induced by TNF α plus IL-17 and IL-1 β was decreased after 24 h of stimulation (Mullins et al. 2009).

In this study, we found that active mites on the surface of the skin equivalents, composed of epidermal keratinocytes and dermal fibroblasts together, resulted in secretion of CTACK, TSLP, IL-1 α , IL-1 β , IL-1ra, IL-6, IL-8, MCP-1, GM-CSF, and M-CSF onto the surface of and, in most cases, into the culture medium in the well below the HSEs (Table 2). Most of these cytokines were measured for the first time in HSEs challenged with scabies mites or extracts. In contrast, mite body extract placed on the surface of the HSEs only induced up-regulated secretion of TSLP and IL-1 α onto the surface and of CTACK into the wells. Therefore, the greatest secretion of cytokines by these HSEs was induced by the burrowing and secretions of live mites.

The notable difference between our HSE data and our previous monoculture experiments is that HSEs secreted IL-1 α , IL-1 β , and IL-1ra in response to live scabies mites on the surface of and burrowing into the HSE, whereas monocultured keratinocytes and fibroblasts secreted little or none of these in response to scabies extract (Table 2). Similarly, we had previously found that HSEs made from cells from two different human skin donors also produced IL-1 α and IL-1 β in response to live scabies mites burrowing on the surface (Table 2; Arlian et al. 1996b). That study only measured IL-1 α and IL-1 β secretion into the medium, whereas in the current study 14 additional cytokines were monitored. Most importantly, IL-1ra was not measured, nor were surface samples collected during that study. These contrasting results between monocultures and HSEs clearly illustrate the importance of cell interactions and the matrix in understanding the influence of a parasite on the host's inflammatory and immune responses to the parasite. However, in the HSE containing both cell types together and a matrix, live scabies mites induced IL-1 α and IL-1 β secretion that was most likely from keratinocytes based on our previous research (Arlian et al. 2003).

The cytokines IL-1 α and IL-1 β in vivo would normally activate cells with IL-1 receptors and therefore promote inflammation. Cells with IL-1 receptors include vascular endothelial cells, T cells, B cells, natural killer cells, macrophages, and neutrophils. IL-1ra is a competitive inhibitor of IL-1. IL-1ra binds to the IL-1 receptor on the cell, and thus blocks IL-1 from binding to the receptor, and this prevents the initiation of the inflammatory reaction. Up-regulating the

production of IL-1ra may be a key first step in the scabies mite's ability to delay the inflammatory reaction that is typically observed during an early scabies infestation. However, it is not clear how the balance between the increased secretion of the proinflammatory IL-1 cytokines and their inhibitor IL-1ra along with the influence of scabies mites and their products on the secretion of other cytokines from these cells and the secretion of cytokines and expression of cellular adhesion molecules from endothelial cells have on the overall response. Because of their proximity to the mite in the skin, keratinocytes and fibroblasts are probably the early responders and play an important part in the host response to scabies mites in the skin. However, scabies extract also down-regulates the expression of the cell adhesion molecules vascular cell adhesion molecule-1 and E-selectin and the secretion of IL-8 by vascular endothelial cells (Elder et al. 2006), and this, together with the increased secretion of IL-1ra by skin cells, may down-regulate the inflammatory response to this parasite.

The scabies molecules that are able to modulate the function of human skin cells and their source are still unknown. The stimulatory molecules that induced the cell responses we observed for the HSEs could have come from mite salivary secretions, fecal material, physical activity of the mite (leg and mouth part digging/crawling motion), molecules released by decomposing mite bodies, and molting secretions released during development. Modulation of the host defenses in blood-feeding insects and mites that feed from the surface of the skin is common and usually associated with pharmacological properties of the saliva (Wikel et al. 1996). Scabies mites are different from these arthropod ectoparasites because they actually burrow into the epidermis to feed and reproduce. However, it is reasonable to assume that selection would have favored a similar adaptation in these mites. Our results suggest that the most likely source of the molecules that modulate fibroblast and keratinocyte function is the salivary secretions or physical activity because the experiments were short-term (48 h) and did not allow sufficient time for significant molting or decomposition of mites that died. One would not expect down-regulation from just physical activity. Evidence for this is that extracts placed on the surface of the HSE, in most cases, did not induce up-regulation of most of the cytokines that were induced by active mites on the surface. Similarly, lack of increased secretion of all cytokines was observed when the extract was placed in the medium that bathed the underside of the HSE. This suggests that salivary secretions or the digging (leg trashing and mouth part movement) that damages the epidermis was primarily responsible for the response.

It appears that early in an infestation, molecules in mite salivary secretions contribute most to modulation of the local keratinocyte and fibroblast responses in the skin. There are generally few mites present early in an infestation. As the population grows, mite numbers increase and mites begin to die as their life expectancy (longevity) is reached or the host defenses kill them. The mite breakdown in the burrow would release many different mite molecules compared with that secreted by live mites. This mite breakdown material is more analogous to the composition of the extract, which contains all soluble body material that was used to stimulate cells in culture. However, the mite body material also contains antigenic molecules that trigger the progressive inflammatory and immune reactions. Evidence for this is that hosts treated for scabies begin to develop strong antibody titers after acaricide treatment when mites die and decompose in the skin. As the amount of antigenic material increases over time, the antigenic stimulus over-whelms the inhibitory influence. In human and animal scabies, significant inflammation in the skin is delayed for weeks and the early scabies-specific antibody titer is low (Arlian et al. 1994, 1995, 1996a; Morgan et al. 1997; Arlian and Morgan 2000).

This research identifies key aspects of understanding the host-parasite interaction in the skin and the delay in clinical manifestation that is characteristic of an early primary scabies infestation. There is increasing interest in developing drugs based on immune modulation to treat many diseases. This research provides some data that are a prerequisite to the possible development of novel approaches to preventing or treating scabies.

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Fig. 1.

Cytokine secretion over time into the well below HSE inoculated with live *S. scabiei* mites on the HSE surface (Mites Top), mites in the lower well (Mites Btm), *S. scabiei* extract on the surface (Ext Top), or extract in the lower well (Ext Btm). Uninoculated HSEs served as controls. Data are from one representative experiment of several replicates and are presented as mean $\pm$ SEM. (Online figure in color.)

Table 1
Cytokine secretion at 48 h (pg/ml) onto the surface of HSEs inoculated with live *S. scabiei* mites on the HSE surface or in the lower well, or with *S. scabiei* extract on the surface or in the lower well

Inoculum	None	Live mites	Live mites	Live mites	<i>S. scabiei</i> extract	<i>S. scabiei</i> extract
Route	Control	HSE surface	Lower well	HSE surface	Lower well	
CTACK	1 ± 1	23 ± 6**	3 ± 2	9 ± 6	36 ± 17	
TSLP	1 ± 1	29 ± 3**	1 ± 0	9 ± 1**	2 ± 1	
GRO α	6,440 ± 390	10,950 ± 1,700	5,750 ± 140	10,360 ± 2,170	8,300 ± 1,180	
TGF α	N.D.	N.D.	N.D.	N.D.	N.D.	
IL-1 α	260 ± 60	850 ± 20**	240 ± 70	430 ± 40**	340 ± 80	
IL-1 β	9 ± 8	39 ± 3**	1 ± 1	6 ± 6	1 ± 1	
IL-1 γ	8,900 ± 860	20,410 ± 1,520**	10,580 ± 1,640	12,410 ± 770	10,140 ± 1,480	
IL-6	4,870 ± 1,740	34,060 ± 3,610**	3,980 ± 2,000	16,340 ± 6,080	13,550 ± 3,460	
IL-8	8,080 ± 2,400	24,700 ± 3,650**	11,000 ± 4,220	19,360 ± 7,210	15,950 ± 4,950	
MCP-1	1,580 ± 350	3,100 ± 370**	1,360 ± 340	3,180 ± 1,100	3,450 ± 860	
G-CSF	1,730 ± 720	4,080 ± 730	1,200 ± 700	5,130 ± 2,220	3,060 ± 980	
GM-CSF	220 ± 90	890 ± 120**	290 ± 190	950 ± 370	320 ± 100	
M-CSF	150 ± 70	750 ± 80**	230 ± 90	220 ± 80	260 ± 90	
VEGF	880 ± 320	1,540 ± 150	490 ± 200	1,370 ± 440	1,300 ± 330	

Uninoculated HSEs served as controls. Data are from one representative experiment of several replicates and are presented as mean ± SEM. N.D., None detected.

** Indicates significant ($P < 0.05$) difference from control.

Table 2
Immune modulation activities of *Sarcoptes scabiei* on normal human skin keratinocytes and fibroblasts in monocultures and skin equivalents

Cell type:	Our previous studies ^{a-e}				This study ^f									
	S. scabiei extract (in media)	Fibroblasts (monoculture)	Skin equivalents (keratinocytes + fibroblasts)	S. scabiei mites (on surface)	Media in well	S. scabiei mites (on surface)	Skin equivalents (keratinocytes + fibroblasts)	S. scabiei mites (in media)	S. scabiei extract (on surface)	Media in well	Media in well	S. scabiei mites (in media)	S. scabiei extract (in media)	S. scabiei mites (on surface)
Inoculum:														
S. scabiei extract (in media)	Media in well	S. scabiei extract (in media)	S. scabiei mites (on surface)	S. scabiei mites (on surface)	S. scabiei mites (in media)	S. scabiei mites (on surface)	S. scabiei mites (in media)	S. scabiei mites (in media)	S. scabiei mites (on surface)	Media in well	Media in well	S. scabiei mites (in media)	S. scabiei extract (in media)	S. scabiei mites (on surface)
CTACK	> ^a	0 ^a	x	>	=	=	=	=	=	=	=	=	=	=
TSLP	> ^a	0 ^a	x	>	=	=	=	=	=	=	=	=	=	=
GRO α	< ^b	x	x	=	=	=	=	=	=	=	=	=	=	=
TGF α	< ^b	x	x	=	=	=	=	=	=	=	=	=	=	=
IL-1 α	= ^c	0 ^c	> ^e	>	=	=	=	=	=	=	=	=	=	=
IL-1 β	> ^c	0 ^c	> ^e	>	=	=	=	=	=	=	=	=	=	=
IL-1 α	< ^c	0 ^c	x	>	=	=	=	=	=	=	=	=	=	=
IL-3	< ^d	x	x	=	=	=	=	=	=	=	=	=	=	=
IL-6	< ^c	$\geq$ ^c	x	>	=	=	=	=	=	=	=	=	=	=
IL-7	0 ^d	x	x	x	x	x	x	x	x	x	x	x	x	x
IL-8	< ^c	$\geq$ ^c	x	>	=	=	=	=	=	=	=	=	=	=
IL-10	> ^d	x	x	x	x	x	x	x	x	x	x	x	x	x
IL-12p7	0 ^d	x	x	x	x	x	x	x	x	x	x	x	x	x
IL-15	0 ^d	x	x	x	x	x	x	x	x	x	x	x	x	x
MCP-1	> ^d	$\geq$ ^d	x	>	=	=	=	=	=	=	=	=	=	=
G-CSF	> ^c	$\geq$ ^c	x	=	=	=	=	=	=	=	=	=	=	=
GM-CSF	> ^d	0 ^b	x	>	=	=	=	=	=	=	=	=	=	=
M-CSF	x	> ^d	x	>	=	=	=	=	=	=	=	=	=	=

Cell type:	Our previous studies ^{a-e}				This study ^f							
	Keratinocytes (monoculture)	Fibroblasts (monoculture)	Skin equivalents (keratinocytes + fibroblasts)	S. scabiei mites (on surface)	S. scabiei mites (on surface)	S. scabiei mites (in media)	S. scabiei mites (on surface)	S. scabiei mites (in media)	S. scabiei mites (on surface)	S. scabiei mites (in media)	S. scabiei mites (on surface)	S. scabiei mites (in media)
	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Surface wash	Surface wash
Inoculum:	<i>S. scabiei</i> extract (in media)	<i>S. scabiei</i> extract (in media)	<i>S. scabiei</i> mites (on surface)	<i>S. scabiei</i> mites (on surface)	<i>S. scabiei</i> mites (in media)	<i>S. scabiei</i> mites (in media)	<i>S. scabiei</i> mites (on surface)	<i>S. scabiei</i> mites (in media)	<i>S. scabiei</i> mites (on surface)	<i>S. scabiei</i> mites (in media)	Skin equivalents (keratinocytes + fibroblasts)	Skin equivalents (keratinocytes + fibroblasts)
Collection site:	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Media in well	Surface wash	Surface wash
VEGF	> ^c	> ^c	x	=	=	=	=	=	=	=	=	=
Eotaxin	0 ^c	0 ^c	x	x	x	x	x	x	x	x	x	x
SCF	0 ^c	0 ^c	x	x	x	x	x	x	x	x	x	x
TARC	> ^a	0 ^d	x	=	=	=	=	=	=	=	x	x
TNF α	0 ^c	0 ^c	x	x	x	x	x	x	x	x	x	x

Data are compared with paired constitutive controls and are scored as equivalent to (=), down-modulated (<), or up-modulated by a moderate (>), or large (>>) amount. Some cytokines were not detected (0), whereas others were not measured (x). SCF, stem cell factor.

^aData taken from Mullins et al. (2009) at 48 h.

^bData taken from Mullins et al. (2009) at 24 h.

^cData taken from Arlian et al. (2003) at 24 h.

^dData taken from L.G.A., Peterson, and M.S.M. (unpublished) at 24 h.

^eData taken from Arlian et al. (1996b) at 16 h.

^fData taken from Fig. 1 or Table 1 of this study.