Expression and Secretion of Leukocyte Chemotactic Cytokines by Normal Human Melanocytes and Melanoma Cells

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The capacity of human melanocytes and melanoma cells to produce IL-8 and monocyte chemotactic and activating factor (MCAF) was investigated. Melanocytes expressed mRNA for IL-8 and MCAF, when stimulated with either IL-1α or TNFα, but not when stimulated with IL-6, IFNγ, or LPS alone. IL-8 and MCAF could be induced in a dose-dependent fashion with doses as low as 0.1 ng/ml TNFα and 0.5 ng/ml IL-1α. IL-8 and MCAF mRNA were rapidly expressed and peaked between 2 and 4 h for IL-8 and between 4 and 8 h for MCAF. This correlated well with the accumulation of IL-8 antigen as measured by a radioimmunoassay. Supernatants from melanocyte cultures stimulated with either IL-1α or TNFα and separated on a heparin-Sepharose column became positive for neutrophil and monocyte chemotactic activity in a dose- and time-dependent fashion. When IFNγ was added to melanocyte cultures stimulated with suboptimal doses of TNFα there was a synergistic increase in secreted IL-8 protein and monocyte chemotactic activity. These data provide further evidence for the possible role of melanocytes in the initiation of an inflammatory reaction. Three different malignant melanoma cell lines stimulated with either TNFα or IL-1α expressed IL-8 mRNA, but not mRNA for MCAF. The IL-8 mRNA signal corresponded well with the amount of secreted IL-8 protein. These data suggest that IL-8 and MCAF may play a role in growth regulation and spreading of melanomas. *J Invest Dermatol 97:593–599, 1991*

The predominant cell type in human epidermis is the keratinocyte, which in recent years has been shown to have immunoregulatory capacities through secretion of cytokines interleukin such as (IL) -1α [1,2], IL-1β [1,2], IL-6 [3], IL-8 [4], tumor necrosis factor (TNF) α [2], TGFα [5,6], TGFβ [7], colony-stimulating factors [8], interferons [9,10], and growth factors [11], either constitutively or upon stimulation. Melanocytes, which represent 2–5% of the cells in the epidermis, have so far been of interest based on their role in pigmentation and in melanomas, whereas little is known of their possible role in inflammation.

Recently, two new families of cytokines have been described of which a member of the first, IL-8, possesses neutrophil chemotactic and activating capacities [12] in addition to T-lymphocyte chemotactic activity [13]. A second group is represented by monocyte chemotactic and activating factor (MCAF), which has been purified [14] and shown to augment monocyte cytostatic activity [14,15]. In addition to chemoattracting monocytes [15], MCAF has been shown to be produced by different tumor cells either upon stimulation or constitutively [15–18] and might, therefore, play a role in tumor growth control by attraction and activation of monocytes at tumor site.

Malignant melanoma cells proliferate vigorously and become highly invasive and metastatic. A protein derived from human melanoma cells that stimulates the growth of melanoma cells was previously isolated and called melanoma growth-stimulating activity (MGSA) [19,20]. Recently, MGSA was cloned [21] and as a member of the same family of cytokines as IL-8 exhibits structural similarities to IL-8 and 44% sequence homology with IL-8 protein [22]. MGSA has in addition been mapped to chromosome 4, at a region that also contains IL-8 [21]. MGSA has been shown to enhance the expression of MGSA mRNA in human melanoma cells and might therefore function as an autocrine growth factor for melanoma cells [21]. It has recently been shown that MGSA has similar neutrophil activating properties as IL-8 and shares the same receptor on neutrophils [23]. Considering the similarities between IL-8 and MGSA, it is therefore of importance to establish whether melanoma cells can produce IL-8. However, although human mela-

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Abbreviations:

BSA: bovine serum albumin
CI: chemotactic index
ICAM-1: intercellular adhesion molecule 1
IFNγ: interferon gamma
IgG: immunoglobulin gamma
IL: interleukin
LPS: lipopolysaccharide
MCAF: monocyte chemotactic and activating factor
MGM: melanocyte growth medium
MGSA: melanoma growth stimulating activity
PBMC: peripheral blood mononuclear cells
PBS: phosphate-buffered saline
PMA: phorbol 12-myristate 13-acetate
PVP: polyvinylpyrrolidone
SSC: standard saline citrate
TGF: transforming growth factor
TNFα: tumor necrosis factor alpha

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nomas are quite often surrounded by a lymphocytic infiltrate, monocytes/macrophages are practically never seen in the surrounding tissue [24,25]. We therefore also evaluated whether human melanoma cell lines produce MCAF.

MATERIALS AND METHODS

Melanocyte Cultures Cultures of normal human epidermal melanocytes (NHEM) were obtained from Clonetics Corporation, (San Diego, CA) and cultured in 75-cm^2 tissue culture flasks (Costar, Cambridge, MA) to subconfluency under serum-free conditions using melanocyte growth medium (MGM) (Clonetics Corporation) containing bovine pituitary extract (0.2% v/v), basic fibroblast growth factor (1 ng/ml), bovine insulin (5 μg/ml), hydrocortisone (0.5 μg/ml), phorbol 12-myristate 13-acetate (PMA) (10 μg/ml), and antibiotics.

Melanoma Cell Lines Metastatic melanoma cell lines A375, C6-14E (IL-1 sensitive) (American Type Culture Collection, Rockville, MD) [14], SK-MEL-2 (American Type Culture Collection, Rockville, MD), and FMX [26] were cultured to subconfluency in Costar 150-cm^2 tissue culture flasks, using 25 ml of RPMI 1640 medium supplemented with 5% fetal bovine serum (Hyclone, Logan, UT), 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 mM L-glutamine at 37°C and 5% CO_2. Cells were incubated with the appropriate stimulus for 18 h before supernatants were collected and kept at −20°C until assayed for IL-8 by RIA.

Northern Blot Analysis of Cultured Cells Melanocytes or melanoma cell lines were grown to subconfluency and stimulated by adding either recombinant IL-1α (rIL-1α, 2×10^7 U/mg, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan), recombinant TNFα (rTNFα, 1×10^7 U/mg, Dainippon Pharmaceutical Co. Ltd., Osaka, Japan), recombinant IL-6 (100 U/ml, Boehringer Mannheim Biochemicals, Indianapolis, IN), recombinant IFNγ (50 ng/ml, 1.2×10^6 U/mg, Biogen Research Corp., Cambridge, MA), or 10 μg/ml LPS (*Escherichia coli,* 055 : B55, Difco, Detroit, MI) to the cultures. Following stimulation, total RNA was extracted as described previously [27]. For the Northern blotting analysis, 5 μg of total RNA from melanocytes or 10 μg of total RNA from melanoma cell lines was denatured and loaded onto each well of a 1% agarose gel. After electrophoresis, mRNA was blotted onto nylon membranes (Schleich & Schuell, Keene, NH). The blots were prehybridized for 16 h at 42°C in Hybrisol I (ONCOR, Gaithersburg, MD) and hybridized with ^{32}P-labeled cDNA probes, which were labeled by random primed DNA labeling kit (Boehringer Mannheim, Indianapolis, IN), at 42°C overnight. Blots were initially washed in 2 × SSC with 0.1% SDS at room temperature followed by high-stringency washes in 0.2 × SSC with 0.1% SDS st 68°C and then exposed to x-ray films (X-OMAT-AR, Kodak, Rochester, NY) with intensifier screens at −80°C for 1–2 d as described previously [27,28]. The following human cytokine DNA were used as probes for hybridization; MCAF cDNA (0.35 kb Pst 1–Pst 1 MCAF coding region) [29], IL-8 cDNA (0.45 kb EcoR1–EcoR1 IL-8 coding region) [30], IL-6 cDNA (0.5 kb Pst 1-Pst 1 coding region), or chicken-β actin cDNA (1.8 kb HindIII–HindIII coding region).

Neutrophil and Monocyte Chemotaxis Assay Leukocytes enriched in monocytes were obtained from the NIH Clinical Center Transfusion Medicine Department (Bethesda, MD) and separated on Ficoll-Hypaque (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). This yielded mononuclear cells consisting of 30–50% monocytes and 50–70% lymphocytes without any neutrophils. Monocyte chemotactic activity was measured using a 48-well multiwell Boyden chemotaxis chamber (Neuro Probe Inc., Bethesda, MD) as previously described [14,31]. Conditioned media obtained from stimulated melanocytes or melanoma cell lines was diluted 1 : 5 in 0.01 M phosphate buffer, pH 7.5, and applied to a 4 × 10 mm heparin Sepharose column (CL-6B; Pharmacia, Uppsala, Sweden). The column was washed with 10 ml of 0.01 M phosphate buffer. The IL-8 and MCAF activities were eluted with 1 ml 1 M NaCl in 0.01 M phosphate buffer. The eluted material was serially diluted in RPMI 1640 medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, 20 mM L-glutamine, and 0.5% bovine serum albumin (BSA) and added in a volume of 26 μl to the lower chamber. Monocytes were resuspended at a concentration of 2×10^6/ml in the medium and 50 μl added to each well in the upper chamber, which was separated from the lower chamber by a polyvinyl-pyrrolidone-free polycarbonate membrane with an 8 μm pore size (Nucleopore, Pleasanton, CA). The chamber was incubated at 37°C and 5% CO_2 for 90 min. The cells that had migrated and adhered to the lower surface of the membrane were fixed in 70% methanol, dried, and stained using Giemsa solution (Fisher Diagnostics, Orangeburg, NY). The migrated cells consisted of more than 98% monocytes by morphology as determined by light microscopy. All assays were performed in triplicate, using coded samples and two high-power fields (400×) were counted for each well. The migration was expressed as chemotactic index (CI), which is the number of migrated cells in presence of conditioned media relative to the number of migrated cells with medium alone.

Neutrophils were obtained from the NIH Clinical Center Transfusion Medicine Department (Bethesda, MD) and separated using a double-layered Ficoll-Paque gradient. The neutrophils were more than 99% pure as determined by light microscopy. Neutrophils were used at a concentration of 1.5×10^6/ml and were allowed to migrate through a PVP-free polycarbonate membrane with a pore size of 3 μm for 45 min before being fixed, stained, and counted as described for the monocyte chemotactic assay.

Radioimmunoassay for Interleukin 8 A rabbit was immunized with rIL-8 [32] 100 μg emulsified initially with Freunds complete subsequently with Freund's incomplete adjuvants, once a week over a 4-week period. Rabbit anti-serum (1 : 450) and culture supernatants (1 : 10) were mixed to a final volume of 500 μl in PBS with 1% BSA. After incubating 30 min at room temperature, ^{125}I-rIL-8 (15,000 cpm) was added to each sample and incubated overnight at 4°C. Five hundred microliters of iron-conjugated goat anti-rabbit IgG (Advanced Magnetics Inc., Cambridge, MA) was added to each sample. After further incubation for 10 min, the samples were pelleted using a magnetic separation unit (Advanced Magnetics Inc.) and washed twice in washing buffer. The amount of rabbit anti-serum and amount of ^{125}I-rIL-8 used was calculated to give a nonspecific binding (3–5%) and a specific binding (30–50%). The titration curve for IL-8 was established to be between 10 pg/ml and 100 ng/ml. All samples were done in duplicate. The specificity of the radioimmunoassay was verified by the inability of recombinant platelet factor 4 and beta thromboglobulin to interfere with the assay at concentrations ranging from 1 ng/ml to 1 μg/ml. Nonimmune rabbit sera failed to bind IL-8.

RESULTS

Expression of IL-8, MCAF, and IL-6 mRNA in Normal Human Melanocytes Normal human melanocytes grown to confluency were stimulated at time 0 with 100 ng/ml of either rIL-1α, rTNFα, rIL-6 (100 U/ml), rIFNγ (10 ng/ml), or LPS (10 μg/ml). These were effective concentrations based on previous experience with keratinocytes, endothelial cells, and fibroblasts [4,28,33]. Total RNA was recovered at 4 h. As seen in Fig 1, both rIL-1α and rTNFα were able to induce IL-8 and MCAF mRNA, but not IL-6 (data not shown). In contrast, neither rIL-6, LPS, nor rIFNγ were effective in inducing the expression of IL-8 and MCAF mRNA (data not shown). The dose-dependent induction of melanocyte-derived IL-8 and MCAF mRNA was studied using confluent melanocyte cultures, which were subjected to variable doses of either rIL-1α (0 to 50 ng/ml) or rTNFα (0 to 100 ng/ml) for 4 h. As seen in Fig 1 both cytokines can induce IL-8 and MCAF, rTNFα inducing the strongest signal for MCAF mRNA. As little as 0.1 ng/ml of rTNFα or 0.5 ng/ml of rIL-1α could induce significant amounts of mRNA for both chemotactic cytokines. As positive controls, human peripheral blood mononuclear cells were stimu-

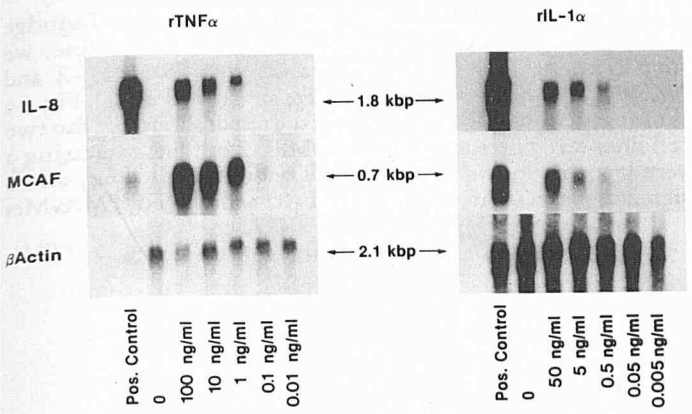

Figure 1. Expression of mRNA for IL-8, MCAF, and β actin in melanocyte cultures stimulated for 4 h with either rTNFα (0.01–100 ng/ml) or rIL-1α (0.005–50 ng/ml). As positive control, LPS (10 μg/ml)-stimulated PBMC (4 h) was used.

Table I. IL-8 Content (ng/ml) in Normal Human Melanocyte Culture Supernatants[a]

	rTNFα	rIL-1α
Concentration (U/ml)		
0	0.86	0.86
0.1	1.1	0.92
1	0.70	0.91
10	2.6	2.8
100	8.2	2.9
1000	11.5	4.1
Timepoints (hours)		
0	0.89	0.89
0.5	1.7	1.9
1	1.6	0.96
2	1.5	0.74
4	3.3	1.0
8	4.8	2.2
12	11.5	4.1

[a] Melanocyte cultures were stimulated with rTNFα (0.01–100 ng/ml) or rIL-1α (0.005–50 ng/ml) for 12 h, or with rTNFα (100 ng/ml) or rIL-1α (50 ng/ml) for different time points. The IL-8 content was detected with a radioimmunoassay. The average of duplicate determinations from a representative experiment is shown.

lated with LPS (10 μg/ml) for 4 h. The kinetics of IL-8 and MCAF mRNA expression in melanocytes was examined by performing Northern blots on total RNA collected at different time points as shown in Fig 2. Both IL-8 and MCAF mRNA were induced within the first hour of exposure to stimulus. Expression of IL-8 mRNA peaked at approximately 2 h when stimulated with rIL-1α and between 2 and 4 h when stimulated with rTNFα, whereas MCAF mRNA peaked at 4 h for rIL-1α stimulation and between 4 and 8 h for rTNFα stimulation. mRNA for both chemotactic cytokines was decreased at 8 h when stimulated with rTNFα and disappeared after 8 h of stimulation with rIL-1α.

Production of IL-8 Antigen by Human Melanocytes To determine whether the induced mRNA for IL-8 was translated and secreted, culture supernatants from confluent melanocyte cultures stimulated for 24 h with cytokines were assayed in a radioimmunoassay for IL-8 antigen. Only melanocyte cultures stimulated with either rTNFα (100 ng/ml) or rIL-1α (50 ng/ml) produced significant levels of IL-8 (11.5 ng/ml and 4.1 ng/ml), whereas rIFNγ, rIL-6, and LPS did not increase the amount of IL-8 above background level (<1 ng/ml). The amount of IL-8 antigen secreted was comparable to the amount produced by keratinocytes, fibroblasts, and large granular lymphocytes, but approximately 10 times less than the amount produced by endothelial cells and monocytes [34,35].

IL-8 antigen was detected in culture supernatants from melanocyte cultures stimulated with either rTNFα or rIL-1α in a dose-related manner (Table I). At doses above 1 ng/ml for rTNFα and 0.5 ng/ml for IL-1α, significant levels of IL-8 could be detected. Confluent melanocyte cultures were further stimulated with either rTNFα (100 ng/ml) or rIL-1α (50 ng/ml) at time 0. Culture supernatants were then collected at different time points and measured for their content of IL-8 antigen. As seen in Table I, a small increase in the antigen content could be detected at 30 min for both rTNFα and rIL-1α, but a significant and time-dependent increase in the IL-8 level was not detected before 4 h.

Analysis of IL-8 and MCAF Bioactivity To study whether the secreted IL-8 was biologically active the melanocyte culture supernatants were also assayed for chemoattractant activity. The above-mentioned culture supernatants were applied to a heparin-Sepharose column. The bound material was then eluted and measured in a microwell chemotaxis assay for neutrophil and monocyte chemotactic activity. As shown in Fig 3*a,b*, both rTNFα- and rIL-1α–stimulated melanocytes generated significant neutrophil and monocyte chemotactic activity in a dose-dependent fashion. When culture supernatants from melanocyte cultures stimulated at time 0 with either rTNFα (100 ng/ml) or rIL-1α (50 ng/ml) were collected at different time points and measured for monocyte and neutrophil chemotactic activity, an increase could be detected over time starting at approximately 2 h (Fig 4*a,b*).

It has recently been shown that when given in combination, rTNFα and rIFNγ could increase the expression of mRNA for the chemotactic cytokines IL-8 and MCAF as well as mRNA for intercellular adhesion molecule-1 in keratinocyte cultures [8]. We therefore investigated whether these cytokines also synergistically stimulated melanocyte cultures. Due to difficulties in obtaining enough material for Northern blot analysis, we stimulated confluent melanocyte cultures with suboptimal concentrations of rTNFα and IFNγ, either alone or in combination and collected supernatant for protein analysis. One ng/ml of rTNFα stimulated melanocyte cultures to produce 2.6 nanogram/milliliter of IL-8, whereas 10 ng/ml of IFNγ alone was unable to do so (920 pg/ml). When the two compounds were administered in combination, a significant increase in antigen content could be detected (5.1 ng/ml), suggesting that TNFα and IFNγ synergize in inducing IL-8. Similar results were seen when the culture supernatants were measured for mono-

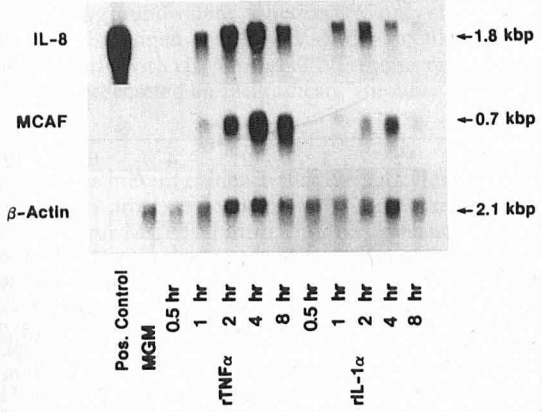

Figure 2. Expression of mRNA for IL-8, MCAF, and β actin in melanocyte cultures stimulated with either rTNFα (1000 U/ml) or rIL-1α (1000 U/ml) for 0.5–8 h. As positive control LPS (10 μg/ml) was used to stimulate PBMC (4 h).

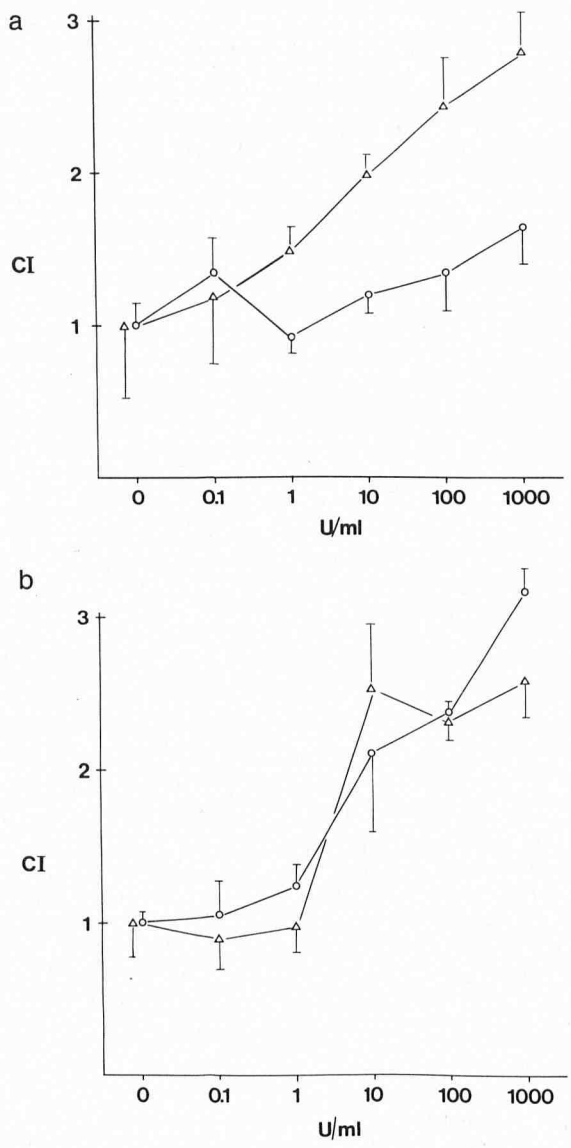

Figure 3. *a*, Detection of neutrophil chemotactic activity (CI) of supernatants from melanocyte cultures stimulated with either rTNFα *(circles)* or rIL-1α *(triangles)* for 24 h and separated by a heparin-Sepharose column. The values are expressed as neutrophil chemotactic activity of stimulated cells relative to the neutrophil chemotactic activity for melanocytes stimulated for 24 h with medium alone. The data expressed are one representative assay of three performed and are expressed as mean of the triplicates ± SEM. *b*, Detection of monocyte chemotactic activity of supernatants from melanocyte cultures stimulated with either rTNFα *(circles)* or rIL-1α *(triangles)* for 24 h and separated by a heparin-Sepharose column.

cyte chemotactic activity (data not shown), suggesting that TNFα and IFNγ also synergize for the induction of MCAF.

Induction of mRNA for IL-8 and MCAF in Melanoma Cell Lines We further studied the ability of three different melanoma cell lines to express mRNA for IL-8 and MCAF. The melanoma cell line A375 C6-14E was grown to confluency and stimulated with either rTNFα or rIL-1α in a dose- and time-dependent manner. As seen in Fig 5a, rIL-1α was able to induce mRNA for IL-8 very rapidly, by 30 min and with doses of rIL-1α as low as 10 U/ml. When stimulated with rTNFα, expression of mRNA for IL-8 could be seen after 1 h and with doses down to 10 ng/ml (100 U/ml). Neither rIL-1α nor rTNFα could induce MCAF mRNA. To judge whether this phenomenon was typical only for this cell line, we stimulated two metastatic melanoma cell lines (SK-MEL-2 and FMX-Met) with either rIL-1α, rTNFα, or LPS. As seen in Fig 6 a similar pattern could be detected. But the responsiveness of the two cell lines was different with the SK-MEL-2 cell line expressing a very low constitutive signal for IL-8 with medium alone, and a significant increase in response to rIL-1α and rTNFα. FMX-Met

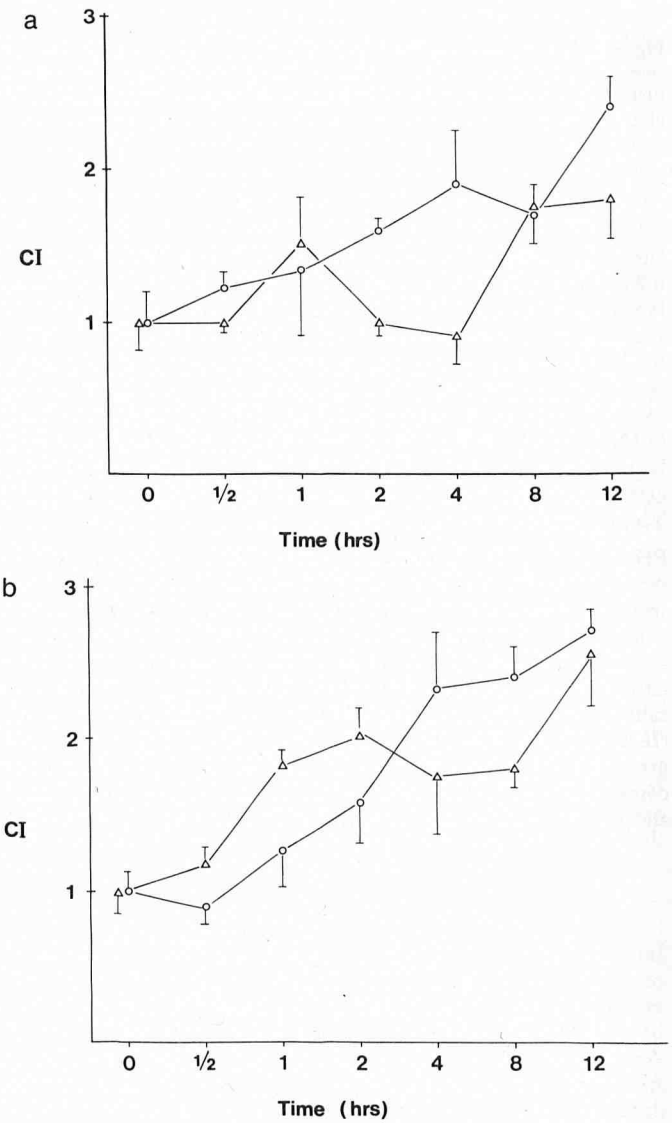

Figure 4. *a*, Detection of neutrophil chemotactic activity (CI) of supernatants from melanocyte cultures stimulated in a time-course manner with either rTNFα (100 ng/ml) *(circles)* or rIL-1α (50 ng/ml) *(triangles)* and separated by heparin-Sepharose column. The values are expressed as neutrophil chemotactic activity of stimulated cells relative to the neutrophil chemotactic activity for melanocytes stimulated for 24 h with medium alone. The data expressed are one representative assay of three performed and are expressed as mean of the triplicates ± SEM. *b*, Detection of monocyte chemotactic activity (CI) of supernatants from melanocyte cultures stimulated in a time-course manner with either rTNFα (100 ng/ml) *(circles)* or rIL-1α (50 ng/ml) *(triangles)* and separated by a heparin-Sepharose column.

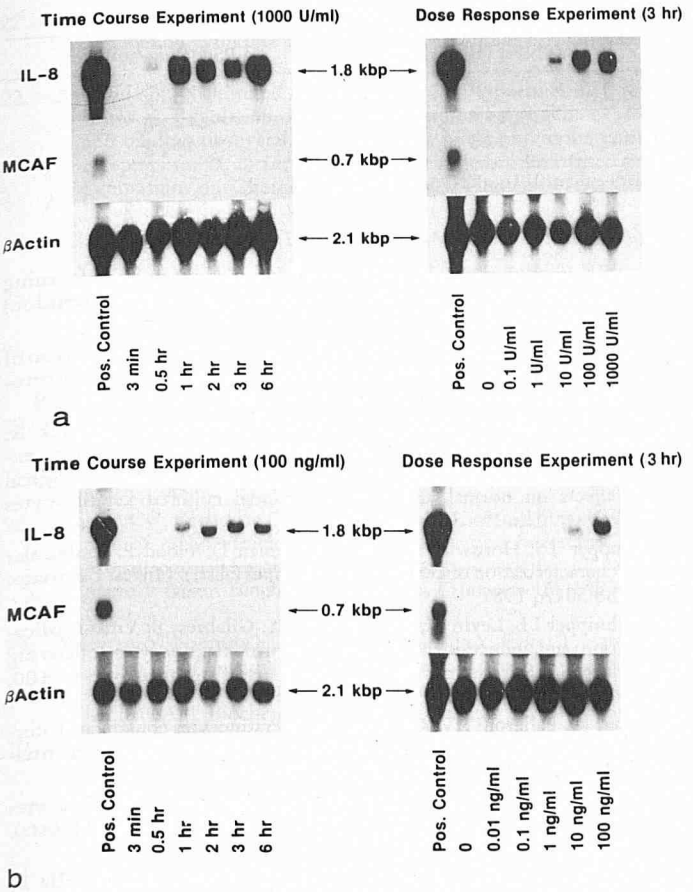

Figure 5. Expression of mRNA for IL-8, MCAF, and β actin as measured by Northern blotting analysis in subconfluent melanoma cell line A375, C6-14E cultures stimulated with rIL-1α *(a)* or rTNFα *(b)* in a time-course manner (3 min–6 h) and in a dose-dependent manner.

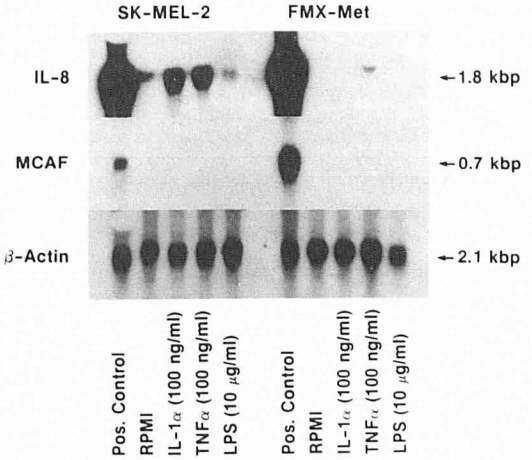

Figure 6. Expression of mRNA for IL-8, MCAF, and β actin as measured by Northern blotting analysis in subconfluent melanoma cell line SK-MEL-2 or FMX-Met cultures stimulated for 4 h with either rIL-1α (100 ng/ml), rTNFα (100 ng/ml), or LPS (10 μg/ml).

could only be stimulated with rTNFα to express IL-8 mRNA. Neither of the two cell lines expressed any signal for MCAF mRNA.

Induction of IL-8 Antigen in Melanoma Cell Line Culture Supernatants When culture supernatants were collected after 24 h and examined in an IL-8 radioimmunoassay, we observed (Table II) that the cell line A375 C6-14E could be stimulated with either IL-1α or TNFα to give a 70–100 times increase in the IL-8 levels over background values, whereas LPS only showed a twofold increase in IL-8 antigen content. SK-MEL-2 could also be stimulated significantly with rIL-1α and rTNFα to secrete IL-8, whereas FMX-Met only secreted an insignificant amount.

DISCUSSION

In this paper we present evidence that melanocytes may participate in inflammatory processes by the production of chemotactic factors such as IL-8 and MCAF. Normal human melanocytes exhibited IL-1α and TNFα-regulated expression of both IL-8- and MCAF-mRNA and bioactivity, as well as IL-8 antigen. The response is specific and selective, as neither LPS, IFNγ, nor IL-6 alone induced the production of IL-8 and MCAF.

It has previously been shown that non-immune cell types like fibroblasts, hepatocytes [4,36], and now melanocytes, in contrast to monocytes, are not able to respond to LPS [12]. Thus, LPS may, via the production of IL-1 and TNF by resident macrophages in the skin, initiate a host response towards intruding microorganisms. This will include the production of IL-8 and MCAF by keratinocytes and melanocytes, which then attract and activate the inflammatory cells.

It has already been reported that keratinocytes can respond synergistically to TNFα and IFNγ, with an increase in mRNA expression for IL-8, MCAF, and ICAM-1 [37]. Melanocytes and melanoma cells, when treated with the two agents in combination, are greatly stimulated to produce fibronectin [38]. Synergy between TNFα and IFNγ has been reported previously in regard to other activities [37–39]. We observed that melanocytes can also respond synergistically to TNFα and IFNγ to produce IL-8 and probably MCAF.

It has recently been shown that upon stimulation with IL-1α, TNFα, and IFNγ, melanocytes increase their expression of ICAM-1 [40] as measured by fluorescence-activated cell sorting. Melanocytes were more sensitive to IFNγ than keratinocytes in their expression of ICAM-1, and IL-1 did not induce ICAM-1 on keratinocytes, whereas melanocytes were able to respond to IL-1. Keratinocytes are not able to express MCAF and only produce IL-8 to a very small extent upon stimulation with TNFα alone [37]. In this paper we show evidence for a greater sensitivity of melanocytes to IL-1α and TNFα. These differences in sensitivity to cytokine induction of IL-8, MCAF, and ICAM-1 may be of importance for the understanding of the role of melanocytes during an inflammatory process. The advantageous location of melanocytes in the skin close to the basement membrane and their multiple dendritic processes may enable them to function as important effector cells in early phases of inflammation.

There is other evidence that melanocytes may play a role in inflammatory responses to external injury induced by trauma, viruses, or bacteria. Melanoma cells and cultured melanocytes contain mRNA for both IL-1α and IL-1β [41]. In addition, melanocytes contain an IL-1β convertase that can act on the keratinocyte-produced 33-kd IL-1β precursor to generate an active 17-kd IL-1β product, which then may act on melanocytes to produce chemotactic cytokines [41].

The failure of IL-6 to induce IL-8 and MCAF expression in human melanocytes is not surprising, because other cell types have not been able to produce IL-8 upon stimulation with IL-6 [36]. This lack of responsiveness is not because melanocytes do not respond to IL-6 at all, as it has been shown that IL-1α, TNFα, and IL-6 are inhibitors of human melanocyte proliferation [42].

Kath et al [43] characterized a series of differences between cultured normal melanocytes and highly malignant metastatic melanoma cells, which included: 1) limited life span for normal melano-

Table II. IL-8 Content in Melanoma Cell Line Culture Supernatants[a]

	RPMI	rIL-1α (50 ng/ml)	rTNFα (100 ng/ml)	LPS (10 μg/ml)
A375 C6-14E	1.25 ng	71 ng	123 ng	2.85 ng
SK-MEL-2	760 pg	13.6 ng	15.5 ng	2.2 ng
FMX	100 pg	80 pg	1.65 ng	46 pg

[a] Amount of IL-8 protein per ml supernatant after stimulation of melanoma cell line cultures with either rIL-1α, rTNFα, or LPS for 24 h.

cytes versus infinite growth for malignant melanoma cells; 2) expression of melanocyte-associated antigens versus expression of melanoma-associated antigens; 3) dependence on exogenous growth factors and other mitogens versus autonomous growth in protein-free medium. The only difference found between advanced primary and metastatic melanomas was that only metastatic melanoma cells proliferated continuously in the absence of growth factors or other proteins.

Cancer cells need to undergo several changes in order to be able to become metastatic. Some of the changes involve alterations in the methylation pattern of the DNA, which then causes genes to be turned on or off. A consequence of this can be the increased ability of tumor cells to grow. This may be caused by growth factors produced by the tumor cells themselves. It has recently been shown that IL-8 can induce haptotactic migration of melanoma cells [44] and thereby increase their motility. In addition, in vivo inoculation of IL-1 augments metastasis of human melanoma cells in nude mice [44] and it has been documented that there is augmented secondary spreading of cancer at sites of inflammation [45]. This secondary enhanced implantation of melanoma cells could be contributed to by locally produced IL-8 by melanoma cells, melanocytes, keratinocytes, endothelial cells, or fibroblasts, when stimulated with IL-1. Unlike normal melanocytes, the melanoma cells no longer can be stimulated to express mRNA for MCAF. MCAF has the ability to attract and activate monocytes to achieve cytostatic capacity [5]. There is a positive correlation between monocyte chemotactic activity of culture supernatants from solid human tumors and the percentage of tumor-associated macrophages [46–48]. The inability of melanoma cells to produce MCAF might account for the reduced number of infiltrating macrophages and thereby influence the tumor growth control. Even though an increasing amount of information has become available on the role of chemotactic cytokines in host defense, their importance in tumor growth control and host surveillance of transformed cells has to be established more precisely in the future.

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