

Monocyte Selectivity and Tissue Localization Suggests a Role for Breast and Kidney-expressed Chemokine (BRAK) in Macrophage Development

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Abstract

Although numerous chemokines act on monocytes, none of them is specific for these cells. Here, we show that breast and kidney-expressed chemokine (BRAK) is a highly selective monocyte chemoattractant. Migration efficacy and *Bordetella pertussis* toxin-sensitive Ca²⁺ mobilization responses to BRAK were strongly enhanced after treatment of monocytes with the cyclic AMP-elevating agents prostaglandin E₂ and forskolin. BRAK is the first monocyte-selective chemokine, as other types of blood leukocytes or monocyte-derived dendritic cells and macrophages did not respond. Expression in normal skin keratinocytes and dermal fibroblasts as well as lamina propria cells in normal intestinal tissues suggests a homeostatic rather than an inflammatory function for this chemokine. In addition, macrophages were frequently found to colocalize with BRAK-producing fibroblasts. We propose that BRAK is involved in the generation of tissue macrophages by recruiting extravasated precursors to fibroblasts, which are known to secrete essential cytokines for macrophage development.

Key words: epithelial tissues • chemokine • prostaglandin E₂ • monocytes • migration

Introduction

Homing of leukocytes to sites of hematopoiesis, antigen priming, immune surveillance, and inflammation largely depends on the presence of chemokines (1–3). Approximately 50 human chemokines are currently known, and this large number reflects the highly complex traffic pattern of precursor and mature blood leukocytes. In broad terms, the chemokines are divided into two functional subfamilies, the inflammatory and the homeostatic chemokines, although several chemokines share both features. Recent progress draws attention to several chemokines with a central role in the basal lymphocyte traffic in extra-lymphoid tissues, such as skin and gastrointestinal tract (for reviews, see references 1–3). Cutaneous T cell-attracting chemokine (CTACK; CCL27) and mucosae-associated epithelial chemokine (MEC; CCL28), two recent CC chemokines with selectivity for the receptor CCR10, are expressed in various epithelial tissues. Thymus and activation-regulated chemokine (TARC) is present in (notably inflamed) skin

tissue and attracts cutaneous lymphocyte antigen (CLA)⁺ T cells more efficiently than CLA⁻ T cells, correlating with expression of CCR4, the receptor for TARC and monocyte-derived chemokine (MDC; CCL22), on CLA⁺ peripheral blood memory T cells. Finally, thymus-expressed chemokine (TECK; CCL25), a chemokine with high expression in intestinal epithelia, selectively interacts with CCR9 expressed on gut-homing T cells. Of note, TARC, MDC, and TECK are also present in lymphoid tissues and attract T cell precursors, indicating that they are not uniquely involved in the control of mature lymphocyte traffic (1–3). Despite remarkable progress in the understanding of lymphocyte migration in epithelial tissues, there is currently no information about chemokines, which control the traffic of monocytes at these locations.

Breast and kidney-expressed chemokine (BRAK; CXCL14) is a recent CXC chemokine with unknown function and receptor selectivity (4–6). The mature sequences of BRAK and its murine ortholog SK1 contain 77 amino acids and are unique with regard to the short NH₂-terminal end of only two amino acids (Ser-Lys), preceding the first of four chemokine-typical Cys residues. There is some

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controversy with respect to the tissues that express this chemokine. BRAK transcripts are highest in human kidney, small intestine, and liver tissues, whereas SK1 transcripts predominate in mouse ovary, brain, and lung tissues but not in murine kidney, intestine, and liver tissues (4–6). Here we demonstrate that BRAK is a highly selective chemokine for blood monocytes. Constitutive expression in normal skin and intestinal tissues suggests a homeostatic rather than an inflammatory role for BRAK, which is possibly related to macrophage development.

Materials and Methods

Reagents and Antibodies. Synthetic chemokines were prepared as described (7). GM-CSF and IL-4 were purchased from Pepro-Tech, prostaglandin E2 from Fluka, and forskolin and LPS from Sigma-Aldrich. MicroBead-labeled mouse anti-human CD4, CD8, CD14, CD20, and CD56 antibodies were from Miltenyi Biotec, and anti-CD3 and S-100 rabbit IgG, and anti-CD20 (L26) and anti-CD68 (PG-M1) mouse monoclonal antibodies were from Dako. Synthetic oligonucleotide primers were from MWG Inc.

Blood Cell Isolation, Cell Culture, and Chemokine Responses. Isolation of human PBLs, monocytes, and neutrophils, and culturing of T cells are described (8, 9). Macrophages were generated by culturing of CD14⁺ monocytes in normal or Teflon foil-coated culture dishes for 5–7 d in macrophage serum-free medium (Life Technologies) with or without 10 ng/ml GM-CSF. The generation of monocyte-derived dendritic cells (DCs) is described elsewhere (10, 11). Stimulation of monocytes with either 1 μ M PGE₂ or 20 μ M forskolin was performed in Teflon foil-coated culture dishes. Chemotaxis and Ca²⁺ mobilization assays are described elsewhere (12).

Northern Blot and PCR Analysis. RNA was extracted from blood leukocytes, tissue culture cell lines, primary cultures of keratinocytes, and dermal fibroblasts or whole tissue sections either by the RNazole B method (Tel-Test Inc.) or by using FP120 FastPrep RNA extraction machine (Savant Instruments Inc.) according to the suppliers instruction. Human epidermis and keratinocytes were prepared from skin tissue (mamma reduction) as described (13). Epidermal single cell suspensions were obtained by trypsin digestion. Northern blot was performed with a ³²P-labeled 314-bp BRAK DNA probe containing the entire coding region (sequence data are available from GenBank/EMBL/DDBJ under accession no. AF073957). The oligonucleotide primers used in reverse transcription (RT)-PCR included those for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; sense ACATCAAGAAGGTGGTGAAGCAGG, and antisense CTCTTCCTTTGTGCTCTTGCTGG; 282-bp fragment, GenBank/EMBL/DDBJ accession no. M33197); β -actin (sense CAGGGCGTGATGGTGGGCATG, and antisense GGCGACGTAGCACAGCTTCTCC; 540-bp fragment, GenBank/EMBL/DDBJ accession no. X00351); BRAK (sense TCCGGTCAGCATGAGGCTCC, and antisense CACCCTATTCTTCGTAGACC; 314-bp fragment, GenBank/EMBL/DDBJ accession no. AF073957); CTACK (sense GAGTCTAGGCTGAGCAACATGAAGG, and antisense GCTTCAGCCCATTTCCTTAGCATCC; 360-bp fragment, GenBank/EMBL/DDBJ accession no. AJ243542); and TECK (sense ATGAACCTGTGGC-TCCTGG, and antisense GGCTCACAGTCCTGAATTAGC; 456-bp fragment, GenBank/EMBL/DDBJ accession no. U86358).

Immunohistochemistry and In Situ Hybridization. Paraffin tissue sections were immunostained with anti-CD68, anti-S-100 (after proteinase digestion), anti-CD3, anti-CD20 (after heat retrieval), and isotype control antibodies by the ABC method (9). For in situ hybridization, ³⁵S- or digoxigenin-labeled sense and antisense mRNA corresponding to position -10 to 304 of BRAK cDNA (GenBank/EMBL/DDBJ accession no. AF073957) was generated and used as described (9). Combined immunostaining and in situ hybridization: initial antibody binding, in buffers treated with diethyl pyrocarbonate containing 5 mg/ml heparin sodium salt (Sigma-Aldrich) and 5 mg/ml nuclease-free bovine serum albumin (Calbiochem), was developed with FastRed (Sigma-Aldrich). After paraformaldehyde fixation and acetylation, hybridization with digoxigenin-RNA probes was performed and detected by NBT/BCIP (Roche).

Results

BRAK Is Selective for Activated Peripheral Blood Monocytes. Freshly isolated peripheral blood leukocytes, including isolated neutrophils, T, B, and NK cells, did not migrate in response to BRAK, as assessed in the modified Boyden chamber chemotaxis assay (not shown). In addition, no activity was found with numerous T cell lines derived from peripheral blood or tonsils, irrespective of their state of activation (short-term activated, proliferating, or resting) or stimulatory conditions (PHA, anti-CD3/CD28 antibodies or superantigen-loaded DCs). However, migration responses were frequently observed with freshly isolated monocytes (37 \pm 21 cells/5 high power fields [HPF]; n = 8) with background migration (in the absence of chemokines) not exceeding 8 cells/5 HPF (Fig. 1 a), suggesting that a subpopulation within the monocyte preparations was attracted by BRAK. Selectivity of BRAK for CD14⁺ monocytes was confirmed in Transwell migration assays with unfractionated peripheral blood mononuclear cells (Fig. 1 b). The 15-fold enrichment in CD14⁺ monocytes is an underestimate due to BRAK-independent (background) migration of CD14-negative cells. Monocyte-derived (immature or mature) DCs and macrophages did not respond. However, short-term treatment of monocytes with a combination of GM-CSF, IL-4, and PGE₂ consistently produced BRAK migration responses, and this observation was further examined.

GM-CSF and IL-4 alone or in combination had no effect whereas stimulation of peripheral blood monocytes with PGE₂ resulted in robust responsiveness to BRAK within 1–3 d of culture, and this effect was lost during prolonged culture (Fig. 1 c). BRAK responses peaked at day 1 or 2 of PGE₂ treatment with 84 to 234 migrated cells/5 HPF (165 \pm 61 cells/5 HPF; n = 8), whereas background migration in the absence of BRAK was negligible (<8 migrated cells/5 HPF). By comparison, monocytes cultured for 2 d in the absence of PGE₂ responded weakly to BRAK (37 \pm 38 cells/5 HPF; n = 7). Of note, marked chemotaxis was only seen at high BRAK concentrations (>100 nM), resembling homeostatic chemokines, which induce chemotaxis at high concentrations as opposed to inflammatory chemokines (1–3). In clear contrast, strong migration re-

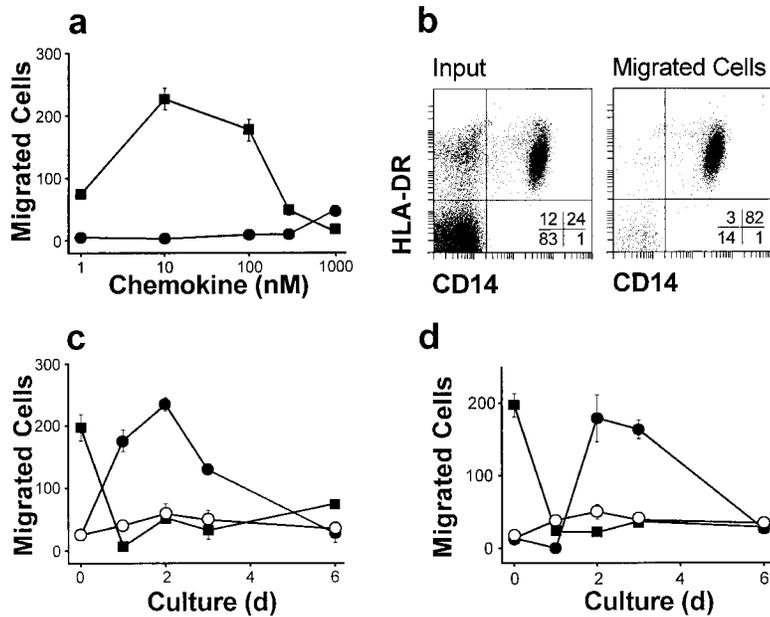


Figure 1. BRAK is a selective chemoattractant for PGE₂- and forskolin-treated monocytes. (a) Peripheral blood monocytes respond weakly to BRAK. Freshly isolated peripheral blood monocytes were examined for chemotaxis responses to BRAK (circles), and MCP-1 (squares) at indicated concentrations. (b) In Transwell chemotaxis assays with freshly isolated peripheral blood mononuclear cells (input cells), BRAK recruited predominantly CD14⁺ monocytes, as assessed by flow cytometric analysis of migrated cells. (c) PGE₂ treatment of monocytes induces strong migration responses to BRAK. Peripheral blood monocytes were cultured in the presence (filled symbols) or absence (open circles) of PGE₂ for indicated number of days and examined for chemotaxis responses to 1 μM BRAK (circles) or 10 nM MCP-1 (squares). (d) Forskolin can substitute for PGE₂ in induction of monocyte responsiveness to BRAK. Peripheral blood monocytes were cultured in the presence (filled symbols) or absence (open circles) of forskolin, and cells were examined for chemotaxis responses to 1 μM BRAK (circles) or 10 nM MCP-1 (squares) at indicated days of culture. Single data point are the mean of triplicate values, each representing the number of migrated cells per 5 HPF.

sponses of freshly isolated monocytes to monocyte chemoattractant protein (MCP-1; CCL2), regulated on activation, normal T cell expressed and secreted (RANTES; CCL5), and stromal cell-derived factor (SDF)-1 (CXCL12) were not maintained during monocyte culture, and lowest migration was consistently seen after 1 d of PGE₂ stimulation (Fig. 1 c, and data not shown). Induction of BRAK responses by PGE₂ was selective for monocytes, as the same treatment of peripheral blood T and B cells did not result in responsiveness to BRAK (not shown). PGE₂ interacts with numerous heptahelical receptors, which activate adenylate cyclase via coupling to heterotrimeric G proteins of the G_s subclass. To determine the requirement for cAMP in induction of BRAK responsiveness, monocytes were examined after culturing in the presence of the adenylate cyclase activator forskolin. Indeed, forskolin was equally effective as PGE₂ in inducing monocyte respon-

siveness to BRAK (Fig. 1 d). Peak migration responses (134 ± 39 cells/5 HPF; n = 4) were obtained at day 2 or 3 of culture, which then declined to base levels by day 6. BRAK responses of cultured but untreated monocytes were moderate (53 ± 33 cells/5 HPF; n = 4). Migration responses to MCP-1 (or RANTES and SDF-1, not shown) were consistently reduced, which fully agrees with the reported PGE₂ effect (14–16). Together, the stimulatory effect of forskolin closely matches our findings with PGE₂ and demonstrates that an adenylate cyclase-dependent mechanism was involved in induction of monocyte responsiveness to BRAK.

BRAK Signals through Bordetella pertussis toxin-sensitive Receptors. In addition to chemotaxis, BRAK induced rapid elevations in the concentration of intracellular free Ca²⁺ in PGE₂-treated monocytes, which is a typical and rapid response to chemokine receptor signaling (Fig. 2). As

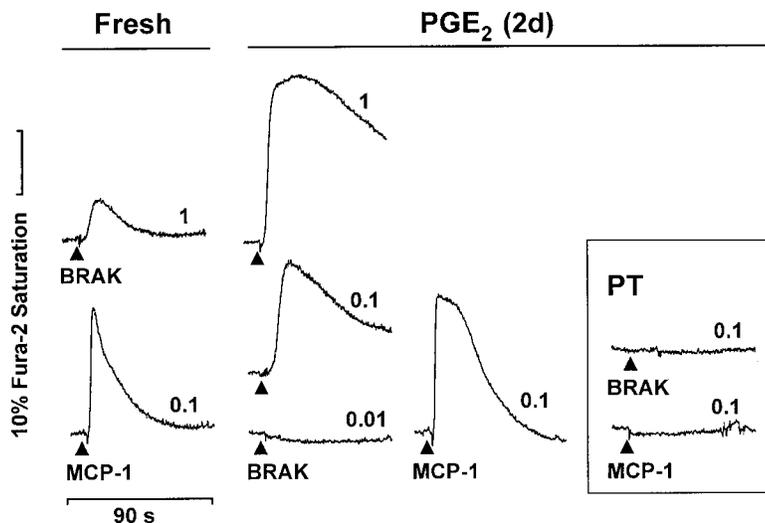


Figure 2. BRAK-mediated elevation of intracellular free Ca²⁺ concentrations in PGE₂-treated monocytes. Freshly isolated monocytes or monocytes cultured for 2 d in the presence of PGE₂ were examined by real-time Fura-2 fluorescence recordings for Ca²⁺ mobilization responses to 0.01–1 μM BRAK and 0.1 μM MCP-1. Preincubation of PGE₂-treated monocytes with PT abolished Ca²⁺ mobilization responses to 0.1 μM BRAK and MCP-1. Arrowheads denote time of chemokine additions.

expected from the chemotaxis data, BRAK-mediated Ca^{2+} signals in freshly isolated monocytes were weak and only detected at 1 μM concentrations. By contrast, both untreated and PGE_2 -treated monocytes showed strong Ca^{2+} -mobilization responses to MCP-1. Onset, rate, and maximal height of BRAK-induced Ca^{2+} signals resemble those induced by MCP-1, whereas restoration of base level intracellular Ca^{2+} concentrations occurred much slower, suggesting a delay in BRAK receptor desensitization. Chemokine receptors couple to heterotrimeric G proteins of the *B. pertussis* toxin (PT)-sensitive $G_{\alpha i}$ subclass (1), and PT sensitivity of the BRAK response demonstrates that the BRAK receptor couples to the same type of signaling ele-

ments. Ca^{2+} -mobilization screening of transfected cells expressing known or putative chemokine receptors failed to identify the BRAK receptor and, together with the unique selectivity of BRAK for monocytes, supports the notion that BRAK interacts with a new as yet unidentified chemokine receptor.

BRAK Expression in Epithelial Tissues. In an RNA dot-blot analysis of 50 human tissues, highest BRAK transcript levels were found in epithelial tissues, foremost in small intestine and kidney, followed by stomach, colon, appendix, and trachea (not shown). Skin RNA was not represented on this blot (see below). Evidently, BRAK expression marks epithelial tissues with prevalent exposure to anti-

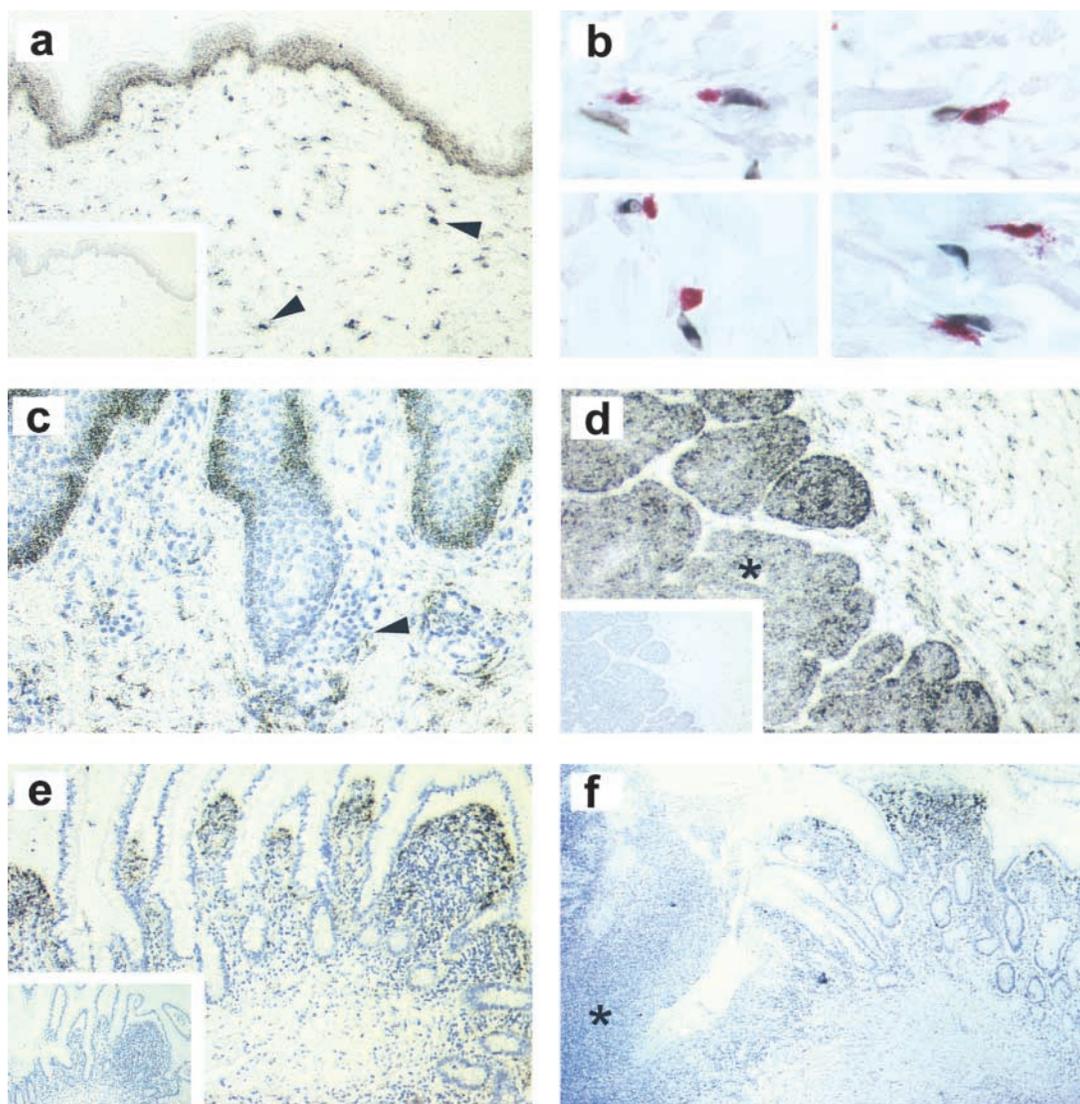


Figure 3. BRAK expression in skin and gut mucosal tissues. In situ hybridization of paraffin-embedded sections using ^{35}S -labeled (a, c–f) or digoxigenin-labeled (b) antisense BRAK RNA probes. (a) BRAK expression (black) in normal skin; arrowheads point at some BRAK-positive cells in the dermis. Control staining with a sense BRAK RNA probe is shown in the inset. (b) Double staining of dermal cells in normal skin with an antisense BRAK RNA probe (black) and anti-CD68 antibodies (red). (c) BRAK expression in skin with atopic dermatitis (arrowhead depicts lymphocytic infiltrate), and (d) basal cell carcinoma (asterisk denotes tumor mass); control sense probe staining in the inset. (e) BRAK transcripts in the small intestine of a healthy individual; control sense probe staining in the inset. (f) BRAK expression in Crohn's disease (asterisk denotes ulcerated tissue). Original magnifications: (f) $\times 40$; (a, d, and e) $\times 100$; (c) $\times 200$; (b) $\times 600$.

gens, and these sites were further studied by *in situ* hybridization analysis. Strong signals with antisense but not sense ³⁵S-labeled BRAK RNA probes were detected in the epidermis of normal skin (Fig. 3 a). Of note, BRAK message was highest in basal keratinocytes of the epidermis and in scattered cells within the underlying dermis. Within the epidermis BRAK signals were most prominent in the basal layer of keratinocytes and were fading out toward the outer layers, indicating that BRAK production diminished with keratinocyte differentiation. In the dermis, BRAK-positive cells were frequently found side-by-side with (CD68⁺) macrophages, as evidenced by *in situ* hybridization with a digoxigenin-labeled BRAK RNA probe in combination with immunohistochemistry (Fig. 3 b). Of 232 ± 29 hematoxylin-positive cells/mm² (total area without perivascular space: 6.3 mm²), 99 ± 16 cells stained positive for BRAK and 77 ± 9 cells stained positive for macrophages, and 20 ± 3% of these macrophages were found to be “paired” with BRAK-positive cells. This phenomenon was not observed with other infiltrated cells, including T cells (CD3), B cells (CD20), and Langerhans cells (S-100) or endothelial cells (CD31) (not shown). Further, T cells, B cells, and DCs represented minor cell populations (each <4 cells/mm²), suggesting fibroblasts as the source of dermal BRAK.

Of interest, in atopic dermatitis (examined in three individuals; Fig. 3 c) and psoriasis (examined in three individuals; not shown) the uniform and strong expression of epidermal BRAK transcripts was interrupted at sites where basal keratinocytes were in contact with inflammatory cells, whereas dermal BRAK expression remained unchanged. Possibly, interaction of keratinocytes with T cells, which predominated the inflammatory infiltrates, caused this marked inhibition. Finally, BRAK expression was prominent in skin sections of two patients with basal cell carcinoma, with strong hybridization signals at the border but fading out toward the center of the tumors (Fig. 3 d), whereas tumor tissue of a patient with squamous cell carcinoma was negative (not shown). Again, dermal BRAK expression was normal in both types of skin tumors.

In contrast to skin, epithelial cells in the small intestine and colon (not shown) did not show any sign of BRAK expression whereas strong hybridization signals were detected in cells within the lamina propria (Fig. 3 e). Here, BRAK-positive cells were more concentrated in the apical as opposed to basolateral parts and were absent in the soft tissue distant from the mucosal epithelia. Of note, CD68⁺ macrophages were also located along the uppermost aspect of the lamina propria near the tips of the villi (not shown). The ulcerated tissue of a patient with Crohn’s disease, including large numbers of mostly B cells and some T cells, as well as adjacent lymphoid follicles did not show signs of BRAK expression (Fig. 3 f). As in normal small intestine, BRAK-positive cells were readily detected in unaffected lamina propria tissue.

BRAK expression in epithelial tissues was further examined by Northern blot and RT-PCR analysis. A single hybridization signal corresponding to an RNA species of 1.8

kb was detected in total RNA extracted from human skin and small intestine tissue (Fig. 4 a). In addition, intact epidermal tissue and trypsin-extracted epidermal cells contained detectable levels of BRAK transcripts whereas human epidermis- and gut epithelia-derived cell lines, including keratinocytes (HaCat), melanoma cells (Hs294T), epidermoid (A431), colon (Caco-2), and cervix (Hela) carcinoma cells, fibrosarcoma cells (HT1080), lung carcinoma cells (A549), embryonic kidney cells (E293), and dermal microvascular endothelial cells (HMEC-1), were negative. Strong BRAK hybridization signals with RNA from epidermis and keratinocyte-rich cell extracts (epidermal extract) as opposed to epithelia-derived cell lines from gut mucosal tissue is in agreement with the results from *in situ* analysis (Fig. 3).

Confirming the Northern blot results, BRAK RT-PCR products are readily observed in RNA from skin, epidermis as well as gastrointestinal tissues (appendix, jejunum, and ileum), as well as primary cultures of epidermal keratinocytes and dermal fibroblasts (Fig. 4 b). Although not detected by Northern blot, HaCat, in submerged cultures resembling undifferentiated keratinocytes, were positive in RT-PCR whereas melanoma-derived HS294T, colon epithelium-derived Caco-2, or PBLs were negative. BRAK cDNA from monocytes was only seen after extended amplification (not shown). PCR amplification of CTACK and TECK cDNA served as a control and confirmed the inverse relationship of their respective RNA expression in skin and gut tissue (1–3).

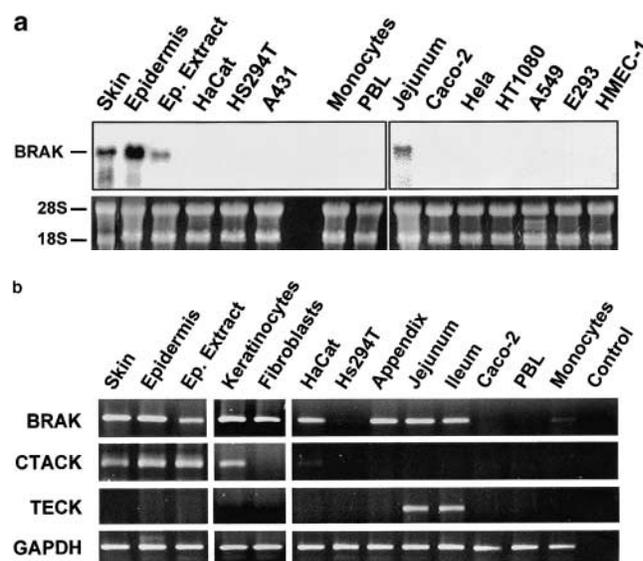


Figure 4. BRAK transcript expression in epithelial cells and fibroblasts. (a) Northern blot analysis of skin and gut tissues, extracted cells, or related cell lines with a ³²P-labeled BRAK DNA probe. Bottom panel, ethidium bromide-stained RNA as agarose gel loading control. (b) RT-PCR analysis of BRAK transcripts. In addition to RNA samples as outlined above, RNA extracted from freshly isolated keratinocytes, primary dermal fibroblast cultures, appendix, and ileum tissues were examined. PCR amplification of GAPDH and β -actin (not shown) cDNA sequences was performed for control of cDNA synthesis.

Discussion

BRAK is not a chemoattractant for peripheral blood T, B, and NK cells or neutrophils. By contrast, BRAK-responsive cells were found to be present among blood monocytes. Furthermore, culturing of monocytes (but not T or B cells) in the presence of PGE₂ resulted in strong migration and Ca²⁺ mobilization responses to BRAK, with peak activities seen between day 1 and 2, followed by a decline to base level activities similar to those observed with freshly isolated blood monocytes. Of note, BRAK is a highly efficient chemokine for PGE₂-treated monocytes (as evidenced by migration indices of >40), exceeding by far the effect seen with a murine histidine-tagged BRAK protein on two human cell lines (6). Monocyte-derived immature/mature DCs and macrophages did not respond to BRAK. We conclude that BRAK is a highly selective chemokine for circulating and PGE₂-treated monocytes.

PGE₂, like many other prostanoids, is a powerful mediator of inflammation, and its production under inflammatory settings as well as its modulatory functions in pyrexia, algisia, and edema are well documented (17). With regard to leukocytes, PGE₂ is both a pro- and antiinflammatory mediator. Relevant to this study are several reports showing that PGE₂ inhibits (rather than promotes) migration and effector functions in neutrophils, monocytes, and lymphocytes (14, 16, 18, 19). Some of these inhibitory effects were shown to be mediated by cAMP, suggesting the involvement of G_s-coupled PGE₂ receptors (14, 16, 20, 21). This is in marked contrast to the PGE₂-induced monocyte responsiveness to BRAK, which also depended on elevated intracellular cAMP levels, as shown with forskolin. As numerous stimuli induce cellular responses via adenylate cyclase activation, PGE₂ may not be the sole physiological agent rendering monocytes responsive to BRAK. An attractive concept for future BRAK studies will center on leukocyte adhesion and transendothelial migration, which involves multiple, in part cAMP-dependent signaling events leading to leukocyte gene expression and differentiation (22, 23). Collectively, the positive effect of PGE₂ and forskolin on monocyte responsiveness to BRAK clearly sets this chemokine apart from other known chemoattractants.

A crucial element for defining the physiological role of BRAK is a detailed understanding of the cellular sources and circumstances (normal versus inflammatory conditions) under which this chemokine is produced. We find prominent BRAK *in situ* hybridization signals in the basal layer of epidermal keratinocytes and dermal cells of skin tissue as well as in lamina propria cells (but not epithelial cells) of colon and small intestine. Expression in basal cell carcinoma, a neoplastic disorder of basal keratinocytes, as opposed to squamous cell carcinoma, fully agrees with undifferentiated as opposed to terminally differentiating suprabasal keratinocytes as the primary source of epidermal BRAK. Presence of BRAK transcripts in submerged primary cultures of keratinocytes and related cell lines was confirmed by Northern blot and RT-PCR analysis. Further, we propose that fibroblasts are the source of BRAK,

as frequency, tissue distribution, and lack of costaining with a digoxigenin-labeled BRAK RNA probe exclude other candidate cell types in the dermis (T and B cells, macrophages, Langerhans cells, endothelial cells). In support, primary dermal fibroblast cultures contained similar levels of BRAK transcripts as primary keratinocyte cultures. Importantly, BRAK-producing cells in the dermis and lamina propria were frequently found in company with macrophages, suggesting that this chemokine marks sites of macrophage differentiation.

Constitutive expression in normal skin and gut tissues qualifies BRAK as a novel member of homeostatic chemokines. However, the powerful effect of PGE₂ in induction of monocyte responses to BRAK does not exclude a role for this chemokine in inflammation. We propose that BRAK is involved in the homeostasis of monocyte-derived tissue macrophages, as IL-6 from dermal fibroblasts was recently shown to determine macrophage differentiation (24, 25). During microbial infections, inflammatory chemokines regulate the recruitment of monocytes to inflammatory sites where PGE₂ induces the transition from refractory to BRAK-responding cells. This change in migratory pattern allows macrophage precursors to colocalize with BRAK-producing fibroblasts for further development into macrophages, thus explaining the frequent colocalization of BRAK-producing fibroblasts with CD68⁺ cells in the dermis. Under normal conditions (in the absence of inflammatory PGE₂), monocytes exit blood circulation constitutively by an unknown but possibly an inflammatory chemokine-independent mechanism. As adhesion and transendothelial migration results in functional and phenotypic changes in leukocytes (22, 23), it is tempting to speculate that extravasation enhances monocyte responsiveness to BRAK. Alternatively, the fraction of BRAK-responsive monocytes already present in blood may enter skin tissue to become a target for fibroblast-derived BRAK, thus allowing their inflammation-independent development into macrophages. In summary, we propose that BRAK regulates the traffic of macrophage precursors at niches in skin and mucosal tissues that support their further development.

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