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Article

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Recruitment of macrophages to sites of cell death is critical for induction of an immunologic response. Calcium concentrations in extracellular fluids vary markedly, and are particularly high at sites of injury or infection. We hypothesized that extracellular calcium participates in modulating the immune response, perhaps acting via the seven-transmembrane calcium-sensing receptor (CaR) on mature monocytes/macrophages. We observed a dose-dependent increase in monocyte chemotaxis in response to extracellular calcium or the selective allosteric CaR activator NPS R-467. In contrast, monocytes derived from mice deficient in CaR lacked the normal chemotactic response to a calcium gradient. Notably, CaR activation of monocytes bearing the receptor synergistically augmented the transmigration response of monocytes to the chemokine MCP-1 in association with increased cell-surface expression of its cognate receptor, CCR2. Conversely, stimulation of monocytes with MCP-1 or SDF-1α reciprocally increased CaR expression, suggesting a dual-enhancing interaction of Ca^{2+} with chemokines in recruiting inflammatory cells. Subcutaneous administration in mice of Ca^{2+}, MCP-1, or (more potently) the combination of Ca^{2+} and MCP-1, elicited an inflammatory infiltrate consisting of monocytes/macrophages. Thus extracellular calcium functions as an ionic chemokinetic agent capable of modulating the innate immune response in vivo and in vitro by direct and indirect actions on monocytic cells. Calcium deposition may be both consequence and cause of chronic inflammatory changes at sites of injury, infection, and atherosclerosis.

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Introduction

Accumulation of immune cells at sites of injury or infection is a critical dimension of host defense that is achieved by highly conserved mediators of cell adhesion and cell motility. The large family of protein cytokines capable of inducing cell migration is collectively termed chemokines. Chemokines can be produced by virtually every cell type in mammals (1–3). Chemokines mediate their function via seven-transmembrane, G protein–coupled receptors (7-TMR); the absence of either chemokines or their receptors results in marked phenotypic alterations in mice (3–5). These include altered inflammatory responses to pathogenic or allergenic challenges, and mitigated atherosclerotic changes in models of vascular disease (6). Extracellular fluids at sites of injury or infection have been reported to contain high concentrations of calcium (7–9), and chronic inflammatory conditions and atherosclerosis are associated with deposition of calcium salts (6, 10, 11). The concentration of calcium in such settings can be substantially higher than in the serum (7–9). We hypothesized that such extracel-lular calcium gradients actively participate in modulating the immune response, acting via the calcium-sensing receptor (CaR).

The CaR is a member of the 7-TMR superfamily, and is responsive to Ca^{2+} concentrations within the millimolar range found in extracellular fluids (12). It was originally defined by its role in mediating systemic calcium homeostasis; however, it has since been shown to have pleiotropic effects including altering cellular proliferation, differentiation, and apoptosis (13–16). In hematopoietic cells, it is expressed on mature monocytes/macrophages and subsets of progenitor populations in the bone marrow (17, 18). Animals engineered to be deficient in this receptor appear normal at birth, but die with severely elevated blood calcium levels within the first few weeks of life (19, 20). Activation of the receptor is maximal at 5 mM Ca^{2+} (13), and selective CaR activators have been developed that efficiently mimic Ca^{2+}-induced activation through an allosteric mechanism (e.g., NPS R-467 and its less active stereoisomer, S-467) (21). These agents are low molecular weight compounds known

as calcimimetics that interact with the CaR's transmembrane domains and potentiate the actions of polycationic agonists (such as Ca^{2+} itself) that bind to the receptor's amino-terminal extracellular domain. Calcimimetics are currently in clinical trials for treating primary hyperparathyroidism, a disorder in which the CaR is underactive, and represent useful pharmacological tools for assessing the CaR's mediatory role in CaR-expressing cells in which high Ca^{2+} modulates cellular function. CaR signal transduction is mediated via a pertussis toxin–inhibitable $G\alpha_i$ pathway as well as a pertussis toxin–insensitive mechanism that probably involves $G\alpha_{q/11}$ (22–24).

We have recently shown cell-surface expression of the CaR on adult human $CD14^+$ PBMC (17, 18, 25). However, the physiological role of the CaR in mononuclear cells is unknown. We examined whether ionic calcium is a chemokinetic agent for human monocytes, whether this activity is mediated via the G protein–coupled CaR, and whether interactions with other chemokinetic agents are induced by CaR activation. We assessed the in vivo consequences of activating the CaR, and noted marked infiltration with monocytes. These data strongly support the role of extracellular calcium in modulating monocyte localization and suggest ionic calcium as a primitive mediator of immune function.

Methods

Preparation of CD14⁺PBMC. Low-density cells were isolated from human and mouse peripheral blood using Ficoll-Hypaque (Pharmacia Biotech Inc., Piscataway, New Jersey, USA). $CD14^+$ monocytes were purified by sorting on a FACSVantage (Becton Dickinson Immunocytometry Systems, San Jose, California, USA), based on CD14 expression. Purified cells were incubated in either calcium-free medium (1× HBSS; Mediatech Inc., Herndon, Virginia, USA) or 1× Iscove's modified Dulbecco's medium (IMDM) (Mediatech Inc.), which contains 1.5 mM Ca^{2+}. The medium was subsequently supplemented with 0.5 mM, 1 mM, 2 mM, 3 mM, or 5 mM $CaCl_2$ (Sigma Chemical Co., St. Louis, Missouri, USA) to achieve the desired level of extracellular calcium, or with 1 μM of the selective CaR activator NPS R-467 or its less active stereoisomer, NPS S-467, in 3 mM Ca^{2+}. Cells were incubated for up to 24 hours in 5% CO_2 humidified air. Monocytes were then detached from the plate using a cell scraper. All calcium concentrations shown in the presented data represent the total Ca^{2+} in the basal medium plus any added Ca^{2+}.

Transmigration assays. Transwells (5-μm pore polycarbonate membrane, 12-mm diameter) (Corning-Costar Corp., New York, New York, USA) were used to assess cell migration using an established methodology (25). The concentration of Ca^{2+} in IMDM in the upper and lower chambers of the Transwell® were adjusted according to a checkerboard analysis of chemotaxis. Calcium concentrations varied from 0–6.5 mM. Purified monocytes (10^4) were then placed in the top chamber with 150 μL of IMDM. Five hundred microliters of the medium was added to the bottom of the well, and was supplemented with varying concentrations of Ca^{2+} or with Ca^{2+} plus 10 ng/mL of MCP-1, 100 ng/mL of SDF-1α, or 10 ng/mL of MIP-1α (PeproTech Inc., Rocky Hill, New York, USA). Cells were incubated at 37°C for 3 hours. To study inhibition of chemotaxis, cells were subsequently incubated with wortmannin (1 μM for 20 minutes at 37°C), herbimycin (1 μM for 20 minutes at 37°C), or genistein (1 μg/mL for 20 minutes at 37°C) before their use in transmigration assays. At the end of the experiment, cells were harvested from the lower chamber and counted using a hemocytometer.

Cytosolic calcium changes. Purified monocytes were loaded with Indo-1 AM (Molecular Probes Inc., Eugene, Oregon, USA). Cells were collected on a FACSVantage® for 30 seconds to establish a baseline emission value for Indo-1 AM. Chemokine ligands were then added to cells incubated in Ca^{2+}-free media, or supplemented with 1.5 mM or 4.5 mM $CaCl_2$ and either the selective calcium receptor activator NPS R-467 or the less active NPS S-467 (1 μM) (a gift of E. Nemeth, NPS Pharmaceuticals Inc., Salt Lake City, Utah, USA). Data acquisition then continued for up to 5 minutes.

Chemokine receptor expression and flow cytometric analysis. Purified monocytes were incubated in Ca^{2+}-free medium supplemented with 1 mM, 3 mM, or 5 mM $CaCl_2$, or with 1.5 mM $CaCl_2$ plus 1 μM NPS R-467 or NPS S-467. Cells were washed once in PBS with 1% FCS and then resuspended in 100 μL of Ca^{2+}-free PBS. Monoclonal antibody against the CaR was added to the cells, and they were incubated alone or with the CaR peptide for 30 minutes at room temperature. Cells were washed with PBS containing 1% FCS, resuspended in PBS with 1% FCS, and incubated for 15 minutes at room temperature with FITC-conjugated anti-mouse IgG. The cells were washed again, and conjugated monoclonal antibody was added and incubated for 15 minutes at room temperature. Monoclonal antibodies used were anti-CXCR4, anti-CCR5 (PharMingen, San Diego, California, USA), anti-CCR2 (R&D Systems Inc., Minneapolis, Minnesota, USA), anti-CD14, and anti-CD4 (Becton Dickinson Immunocytometry Systems). Stained cells were fixed with 1% paraformaldehyde and were assayed within 24 hours. Flow cytometric analysis was performed using a dual laser FACSCalibur calibrated with 2-μm CaliBRITE beads (both from Becton Dickinson Immunocytometry Systems). Data acquisition and analysis were performed using CellQuest software (Becton Dickinson Immunocytometry Systems).

Breeding and genotyping of CasR⁻/⁻ mice. $CasR^{-/-}$ mice were bred as described previously (19). To produce $CaR^{-/-}$ mice for this study, heterozygotic mice were intercrossed. The mice studied were of either 129S6/SvEv or 129S6/SvEv/Swiss Webster mixed genetic background. (Mice homozygous for the *CaR* knockout allele do not live longer than 3 weeks.) Mice were genotyped using the following protocol. A sample
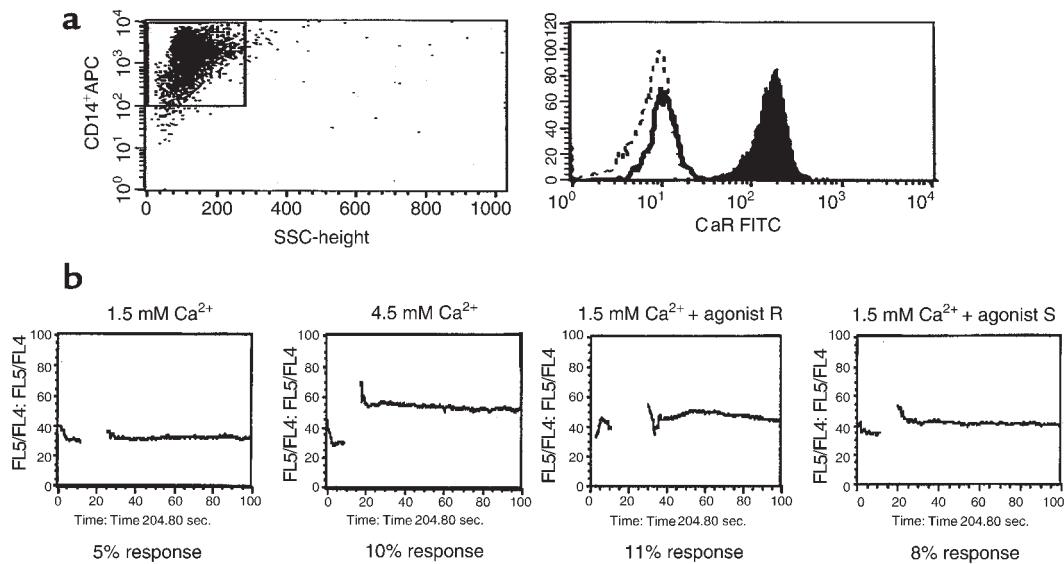
Figure 1
(**a**) CD14⁺ monocytes stain positively for the CaR, and binding of anti-CaR antibody is inhibitable by preincubation with CaR peptide. Purified CD14⁺ PBMC (scattergram: vertical axis is log fluorescence with allophycocyanin-conjugated anti-CD14; horizontal axis is side scatter) were exposed to anti-CaR antibody (right panel; solid histogram) or isotype control (open histogram) and examined by flow cytometry. Monocytes were also preincubated with CaR peptide before staining with anti-CaR antibody (dashed histogram). Veritical axis is number of events; horizontal axis is log fluorescence with fluorescein isothiocyanate–conjugated anti-CaR. Data represent one of ten independent experiments with comparable results. (**b**) Elevating the extracellular Ca^{2+} concentration or adding the selective CaR activator NPS R-467 induces elevation of cytosolic Ca^{2+}. Sustained increases in cytosolic Ca^{2+} were seen in response to 4.5 mM Ca^{2+}, or 1 μM NPS R-467 or S-467 in the presence of 1.5 mM Ca^{2+}. All calcium concentrations shown represent the total calcium content of the basal medium plus added calcium. Data are from one of three independent experiments with similar results.

of DNA (1 μL) was obtained from a section (< 5 mm) of the tail of each mouse to be genotyped according to established techniques (19). In this way the genotypes of *CaR⁻/⁻*, *CaR⁺/⁻*, and *CaR⁺/⁺* mice were obtained. Peripheral blood was obtained from mice of each genotype at the time of sacrifice on day 6–8 after birth.

Biologic activity of ionic calcium in vivo. C57BL/6 mice (The Jackson Laboratory, Bar Harbor, Maine, USA) were injected subcutaneously at a marked, shaved spot on the abdomen with 20 μL of 5 mM $CaCl_2$ or 20 μL of MCP-1 at a concentration of 10 ng/mL, with a combination of either MCP-1 and 5 mM $CaCl_2$, or 10 μM NPS R-467 in PBS. Control mice were injected subcutaneously with 20 μL PBS alone. All subcutaneously injected agents contained less than 0.004 ng/mL of LPS by the limulus assay (Sigma Chemical Co.), as described previously (25). Mice were sacrificed by CO_2 asphyxiation 18 hours after injection, and injection sites were excised and snap frozen. From these, 4-μm cryosections were obtained and stained with hematoxylin and eosin. Immunocytochemistry was performed on the frozen sections using alkaline phosphatase–conjugated Mac-1 antibody (PharMingen) as described previously, and assessed by light microscopy (25).

Results
Calcium mobilization and chemotaxis. Combining flow cytometry results with our prior data showing the presence of CaR mRNA and protein in monocytes by RT-

PCR and Western analysis, we demonstrated that the CaR is expressed on the cell surface of PBMC (Figure 1a). We next examined the CaR's functional relevance in monocytes. Extracellular Ca^{2+} increased the cytosolic Ca^{2+} concentration in a dose-dependent manner, with a maximal response at 4.5 mM Ca^{2+} (Figure 1b), consistent with the receptor's known capacity to elevate cytosolic calcium by activating phospholipase C.

A transmigration assay was then used to determine if calcium was capable of inducing monocyte chemotaxis. A dose-dependent effect was seen, with maximal chemotaxis observed at 4.5 mM Ca^{2+} in a checkerboard analysis of chemotactic response to a concentration gradient of ionic calcium. Chemotaxis was inhibitable by pertussis toxin and by the tyrosine kinase inhibitors herbimycin and genistein, but not by the PI-3 kinase inhibitor wortmannin (Figure 2, a and b). The role of the CaR in these responses was further assessed by exposure of the cells to spermine, a polycationic CaR agonist (26), and to the selective CaR activator NPS R-467 or its less active stereoisomer, NPS S-467 (21, 27). Stimulation of cytosolic calcium responses and transmigration were noted with each of these agents. The greater potency of R-467 than S-467 in enhancing the actions of high Ca^{2+} on these two biological responses is fully in accord with their known pharmacological actions on the cloned CaR, and strongly support the receptor's role in mediating these actions of extracellular Ca^{2+} on monocytes (21) (Figure 2c).

a

Top chamber [Ca²⁺] / Bottom chamber [Ca²⁺]	0 mM	2 mM	3.5 mM	4.5 mM	6.5 mM
0 mM	120 +/- 16	130 +/- 42	200 +/- 40	200 +/- 30	600 +/- 45
2 mM	630 +/- 78	140 +/- 23	300 +/- 29	360 +/- 36	680 +/- 75
3.5 mM	1,100 +/- 273	480 +/- 65	180 +/- 25	480 +/- 61	720 +/- 84
4.5 mM	1,800 +/- 350	790 +/- 61	460 +/- 56	200 +/- 30	680 +/- 74
6.5 mM	670 +/- 82	1,350 +/- 125	780 +/- 92	670 +/- 73	180 +/- 25
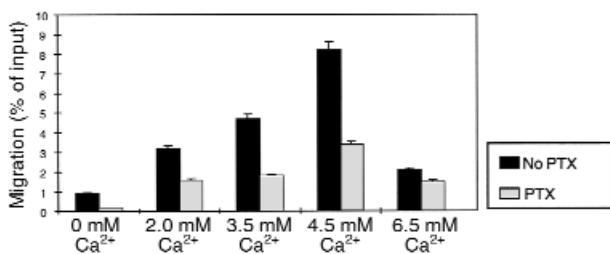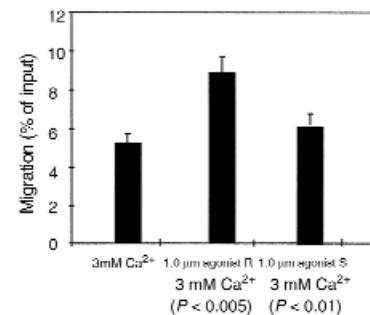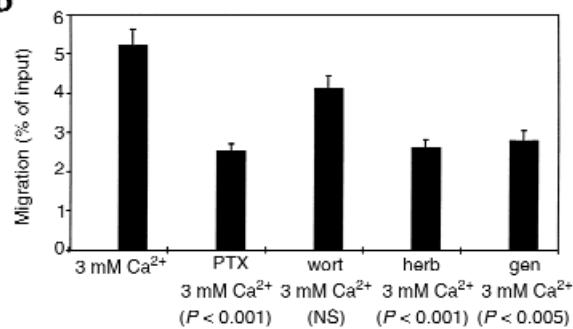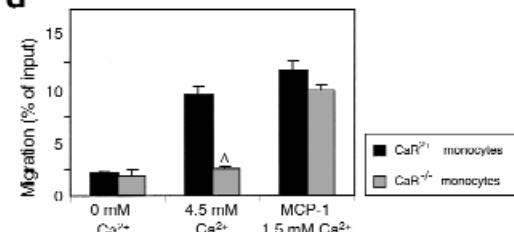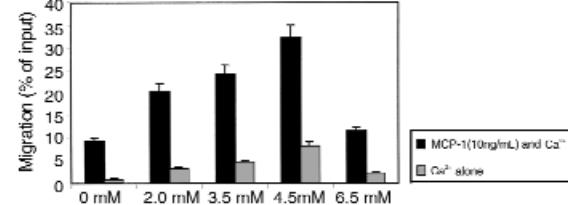
Figure 2

Monocytes migrate toward Ca^{2+} in a dose-dependent manner that is inhibitable by pretreatment with pertussis toxin, genistein, or herbimycin, and is potentiated by the selective CaR activator NPS R-467 and the chemokine MCP-1. (**a**) Transmigration assays were used to determine if Ca^{2+} was capable of inducing monocyte chemotaxis within a checkerboard analysis (upper panel). An input of 10^4 monocytes was used, and the mean numbers of cells migrating in response to each Ca^{2+} gradient are shown for three independent experiments. A dose-dependent effect was seen, with maximal chemotaxis observed at 4.5 mM Ca^{2+}, which was inhibitable by pretreatment with pertussis toxin (PTX) (lower panel). (**b**) Chemotaxis of monocytes toward a positive gradient of Ca^{2+} at 3 mM was also inhibitable by pretreatment with the tyrosine kinase inhibitors genistein (gen) or herbimycin (herb), but not by the PI-3 kinase inhibitor wortmannin (wort). (**c**) The role of the CaR in mediating the chemotactic response to elevated Ca^{2+} was supported by the greater potency of R-467 (agonist R) than of S-467 (agonist S) in stimulating chemotaxis. It should be noted that no specific antagonists or neutralizing antibodies for the CaR have been defined to date. P values represent comparison with 3-mM Ca^{2+} control using the Student's t test. (**d**) Chemotaxis of PBMC obtained from $CaR^{-/-}$ mice and $CaR^{+/+}$ mice also evaluated in response to 4.5 mM Ca^{2+} and to MCP-1 in the presence of 1.5 mM Ca^{2+}. The percent migration of $CaR^{+/+}$ monocytes (black bars) and $CaR^{-/-}$ monocytes (gray bars) are shown. The data from two separate experiments are shown (mean ± SEM). $^AP = 0.0481$, Student's t test. (**e**) Chemotaxis of $CD14^+$ monocytes toward a positive concentration gradient of MCP-1 was also dependent upon and potentiated by Ca^{2+}. Migration of monocytes in the presence of varying levels of Ca^{2+} with MCP-1 (black bars) or without MCP-1 (gray bars) is shown. The percentage of cells migrating in response to MCP-1 could be augmented by increased Ca^{2+} concentrations with peak added activity at 4.5 mM Ca^{2+}. Data represent mean ± SEM of at least three independent experiments.

To confirm the role of CaR in mediating chemotaxis induced by a calcium gradient, we examined mononuclear cells derived from neonatal $CaR^{-/-}$, $CaR^{+/-}$, and $CaR^{+/+}$ mice. The cells were capable of chemotactic movement toward MCP-1; however, $CaR^{-/-}$ cells failed to transmigrate to the Ca^{2+} gradient (Figure 2d), definitively demonstrating the role of the CaR in the response.

Chemokine receptor interactions. We next evaluated whether calcium interacted with other regulators of monocyte migration by assessing the coexpression of chemokine receptors and the CaR on primary human adult monocytic cells. CCR2 and CCR5 were present on 100% of $CD14^+$ PBMC, and CXCR4 was present on 87%; coexpression of the CaR with CCR2 was noted in 97% of $CD14^+$ PBMC, and coexpression with CXCR4 was seen in 83%. Functional responses of monocytes to activation of chemokine receptors were then measured in the presence or absence of CaR stimulation using

transmigration assays. Chemotaxis of CD14$^+$ PBMC to a positive concentration gradient of MCP-1 was dependent upon extracellular calcium, as has been reported by others (28, 29), whereas MIP-1α and SDF-1α induced migration independent of calcium (data not shown). The percentage of cells migrating in response to MCP-1 could be augmented by raising extracellular Ca^{2+}, with peak activity noted at 4.5 mM Ca^{2+} (Figure 2e). The increase in transmigration elicited by a combination of MCP-1 and elevated Ca^{2+} concentrations was greater than the sum of transmigration evoked by either MCP-1 or Ca^{2+} alone, indicating the synergistic nature of the interaction. In contrast, no synergistic effect was noted when either MIP-1α or SDF-1α was used in conjunction with added Ca^{2+}.

The mechanism by which Ca^{2+} enhanced responsiveness to MCP-1 was evaluated by assessing receptor expression kinetics after CaR stimulation. Exposure of cells to elevated Ca^{2+} resulted in upregulation of cell-surface CCR2 at 3 hours (Table 1). In contrast, antibodies directed against CCR5 failed to demonstrate any change in median fluorescence intensity, whereas CXCR4 expression increased but did not achieve statistical significance. Similarly, the fluorescence intensity of anti-CD4 antibody staining did not vary with Ca^{2+} concentration, thereby excluding a nonspecific enhancement of antibody staining in the presence of increased calcium. Inhibition of new protein synthesis by cycloheximide had no effect on the Ca^{2+}-mediated upregulation of CCR2, indicating that Ca^{2+} altered receptor processing or trafficking (or both) between cell-surface and intracellular pools, rather than the generation of new receptor molecules.

To assess whether there was reciprocal regulation of the CaR by chemokine receptors, we evaluated CaR surface expression on cells before and after exposure to the chemokines SDF-1α or MCP-1. The mean fluorescence intensity of CaR expression was 361 ± 25 units (mean ± SEM) for untreated monocytes, and rose to 673 ± 59 after MCP-1 stimulation. Monocytic expression of CaR rose to 519 ± 48 with SDF-1α treatment.

Table 1
Exposure of CD14$^+$ monocytes to Ca^{2+} enhances CCR2 expression in a dose-dependent manner

	Mean fluorescence intensity of CCR2	
	Mean ± SEM	P value
0 mM Ca^{2+}	204 ± 16	-
1.5 mM Ca^{2+}	278 ± 39	0.0045[A]
2.5 mM Ca^{2+}	296 ± 35	0.0007[A]
4.5 mM Ca^{2+}	307 ± 35	0.0003[A]
1.5 mM $^{Ca2+}$ and agonist R	460 ± 52	0.0135[B]
1.5 mM $^{Ca2+}$ and agonist S	417 ± 48	0.0205[B]

Monocytes were exposed to varying concentrations of Ca^{2+} and to the selective CaR activator NPS R-467 (1 μM) or NPS S-467 (1 μM) in the presence of 1.5 mM Ca^{2+}. The median fluorescence intensity for CCR2 expression was measured for each Ca^{2+} concentration and in the presence of NPS R-467 or NPS S-467. Mean values ± SEM from three independent experiments are shown. [A]Student's *t* test vs. 0 mM Ca^{2+}; [B]Student's *t* test vs. 1.5 mM Ca^{2+}.

In vivo monocyte migration toward calcium. To determine whether Ca^{2+} was active as a chemoattractant for monocytes in vivo, Ca^{2+} alone (5.0 mM), MCP-1 alone, or both Ca^{2+} and MCP-1 were injected subcutaneously into C57BL/6 mice, and the tissue was excised 18 hours later. Accumulation of mononuclear cells staining positive for the monocytic marker Mac-1 was noted in mice injected with either Ca^{2+}, MCP-1 (Figure 3), or 10 μM NPS R-467. No subcutaneous infiltrates of cells were detected in mice injected with PBS alone. The greatest degree of monocyte infiltration into subcutaneous tissues was seen with 5 mM Ca^{2+} plus MCP-1, supporting in vivo the synergistic relationship observed in vitro (Figure 3). No significant infiltrate of neutrophils into the site of subcutaneous injection was seen under any of the conditions used, as determined by hematoxylin and eosin staining of skin sections.

Discussion

Ionic calcium is highly regulated in vivo, yet can be elevated in specific tissue microenvironments such as the bone marrow, and is particularly altered in the context of ongoing cell death and inflammation. Hypothesizing that this phenomenon may serve as a primitive regulator of immune cells, we examined a number of hematopoietic cell types for expression of the CaR. Whereas this receptor is expressed on primitive hematopoietic cells (I. Olszak, manuscript in preparation), it is strikingly abundant on mature monocytic cells and is therefore a candidate mediator of immunologic function of this member of the innate immune system.

Localization of monocytes/macrophages at sites of injury or inflammation is crucial for initiation of their role in host defense, and has been demonstrated to be regulated by a number of members of the chemokine family. The CaR is a seven-transmembrane-spanning and G protein–coupled receptor that has been shown to have a number of effects on cell physiology (13). A single prior report has indicated that the murine bone marrow stromal cell line ST-2 is capable of chemotaxis in the context of CaR stimulation (18). Whether this molecule alters the motility of primary cells or cells of the immune system had not previously been addressed.

We documented the responsiveness of monocytes to signaling through the CaR in a Gα_i-coupled pathway mediated by tyrosine kinase, but not PI-3 kinase. The activation of this receptor by either polycationic cognate ligands (e.g., high Ca^{2+} or spermine) or NPS R-4E7, an allosteric activator of the CaR, resulted in the transmigration of monocytic cells, providing a mechanism to explain the prior observation of others that calcium may enhance the migration of monocytes to oxidized LDL (30). The absence of chemotaxis of *CaR$^{-/-}$* monocytes toward Ca^{2+} even though they responded to a control chemokine shows the specificity of the phenomenon for the CaR. Conjecturing that the Ca^{2+} signal would probably
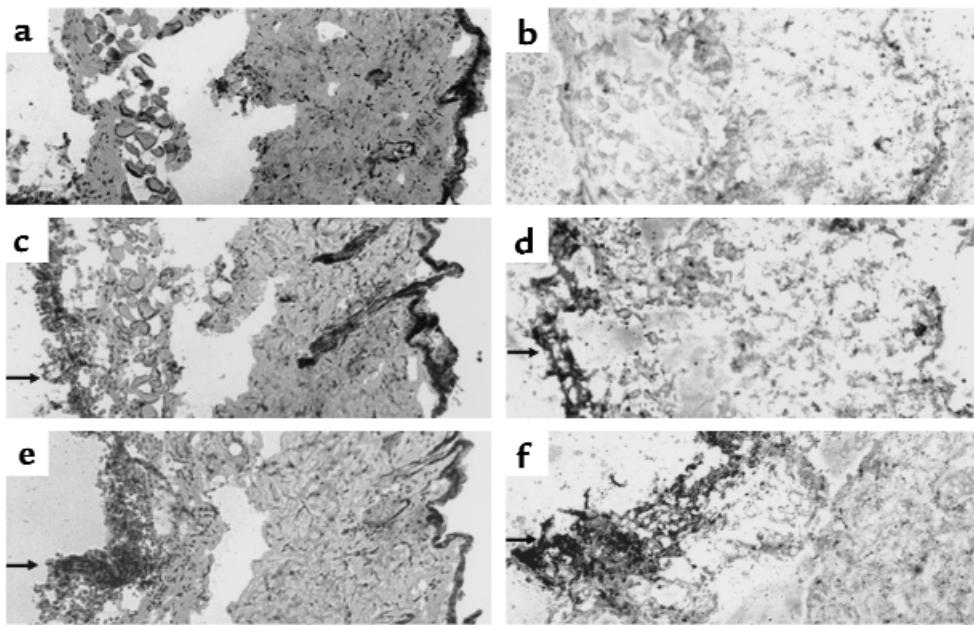
Figure 3
Ca^{2+} is a chemoattractant for monocytes in vivo. Frozen sections of skin were obtained after subcutaneous injection of mice with PBS alone (**a** and **b**), 5.0 mM $CaCl_2$ alone (**c** and **d**), or 5.0 mM $CaCl_2$ plus 10 ng/mL MCP-1 (**e** and **f**). Sections were stained with hematoxylin and eosin (**a**, **c**, **e**) or alkaline phosphatase–conjugated, macrophage-specific Mac-1 antibody (**b**, **d**, **f**). Occasional cells in skin from mice injected subcutaneously with PBS alone stained positively for Mac-1. However, $CaCl_2$ alone and the combination of $CaCl_2$ plus MCP-1 caused a dense infiltrate of mononuclear cells that stained positively for Mac-1 (indicated by arrows in **c**, **d**, and **f**). Data are representative of one of two independent experiments.

interact with other mediators of cell response, we assessed whether the migratory response to MCP-1 was similarly modulated by CaR activation. The impact of CaR signaling was manifested in upregulation of MCP-1 receptor, mediated by a mechanism of altered intracellular trafficking rather than new protein synthesis. A reciprocal increase in CaR expression was noted with stimulation of CCR2 with MCP-1, documenting a clear interrelationship of these two unrelated members of the 7-TMR family. This interaction was evident in vivo as well as in vitro when ligands were used in isolation or in combination in animals, and generated marked monocytic infiltrates at the site of injection. Thus ionic calcium serves to directly and indirectly influence monocyte migratory response. It can play a role in the tissue localization of primary monocytes, and may potentiate protein-mediated induction of chemotaxis. The contribution of this signal to inflammation is dependent upon local calcium concentrations, and therefore may be relevant for settings of either extensive injury or in the context of chronic inflammation.

The CaR induction of monocyte infiltration provides insight regarding the persistence of inflammation at sites of prior injury subsequent to resolution of the initiating event. The presence of local calcium may further monocytic recruitment, thereby perpetuating inflammatory infiltration. Sites of local accumulation of calcium such as atherosclerotic plaques, granulomata, calcific tendinitis, or calcium pyrophosphate disease (pseudogout) may provide reservoirs of calcium ions that serve to encourage monocyte localization. Interruption of this chemokinetic stimulus through specific inhibition of the CaR may provide a therapeutic opportunity in these disease contexts.

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