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Transformation-Associated Cytokine 9E3/CEF4 Is Chemotactic for Chicken Peripheral Blood Mononuclear Cells

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9E3/CEF4, which is released from transformed chicken embryo fibroblasts (CEF), is a member of the platelet factor 4 family of inflammatory proteins and may be the avian homolog of interleukin-8. Since the function of 9E3/CEF4 is unknown, we examined the effect of the protein on mitogenicity and chemotaxis, as well as its expression, in fibroblasts and peripheral blood cells. 9E3/CEF4 mRNA was expressed in chicken peripheral blood monocytes, and its expression was stimulated by incubation of the monocytes with lipopolysaccharide or phorbol myristic acetate. Boyden double-membrane analysis of chemotaxis showed that 9E3/CEF4 was chemotactic for chicken peripheral blood mononuclear cells, as well as for heterophils. Untransformed CEF and CEF transformed with Rous sarcoma virus also migrated to 9E3/CEF4 protein, as measured by Boyden single-membrane analysis. 9E3/CEF4 was slightly mitogenic for CEF, causing a doubling of [^{3}H]thymidine uptake when added to serum-starved CEF. 9E3/CEF4 was found associated not only with the cell and in the culture medium of Rous sarcoma virus-transformed CEF but also with the extracellular matrix. The in vivo role of 9E3/CEF4 may be involved with chemotaxis and metastasis, rather than with direct stimulation of mitogenicity.

9E3/CEF4 is a chicken gene constitutively expressed in chicken embryo fibroblasts (CEF) transformed by Rous sarcoma virus (RSV) (5, 44) and other oncogenic viruses and which can be induced in nontransformed CEF by inflammatory and mitogenic stimuli (3). It is a member of a family of small secretory proteins related to platelet factor 4 (PF4), which may mediate the inflammatory response.

The PF4-related proteins are expressed in a variety of cell types, including platelets, endothelial cells, lymphocytes, keratinocytes, synovial cells, and monocytes (43). Several functions have been ascribed to members of the PF4 family. PF4 inhibits angiogenesis (23) and is a chemoattractant for neutrophils, monocytes (11), and fibroblasts (41). Monocyte chemotactic peptide/monocyte chemoattractant factor is a chemoattractant for monocytes (28, 48). Interleukin-8 (IL-8), the most thoroughly defined member, is chemotactic for neutrophils (45) and T cells (20), induces granule secretion and lipoxygenase product release from neutrophils (22, 37), and modulates neutrophil adherence to endothelial cells (7). All of these functions are compatible with a role for the PF4-related proteins in inflammation and wound healing.

Less understood is the potential role of the PF4-related proteins in transformation. 9E3/CEF4 is 51% homologous to IL-8 at the amino acid level and 45% homologous to gro/melanoma growth-stimulatory activity (gro/MGSA) (43) and is likely to be the chicken homolog of one of these. Both of these proteins are chemotactic for neutrophils (39, 45), and gro/MGSA has been reported to be mitogenic for melanoma cells (33, 34), making it tempting to speculate that the stimulation of growth by these peptides could be responsible for the increased cell growth of tumor cells.

In order to define a function for 9E3/CEF4, we examined the ability of purified 9E3/CEF4 protein to stimulate chemotaxis and mitogenicity in chicken peripheral blood cells and fibroblasts. We found that, while 9E3/CEF4 is slightly mitogenic for fibroblasts, its stronger activity is as a chemoattractant.

MATERIALS AND METHODS

Isolation of chicken peripheral blood cells. Chicken peripheral blood was centrifuged on a two-step gradient of Histopaque-1119 and Histopaque-1077 (Sigma Chemical Company, St. Louis, Mo.), in accordance with the manufacturer's directions. The top band, which contained monocytes and lymphocytes, was removed and washed twice with phosphate-buffered saline (PBS), and the cells were cultured for 2 h in Dulbecco minimal essential medium with 10% serum. The nonadherent cells were removed, recultured to remove residual adherent cells, and used as the lymphocyte fraction. The monocyte fraction was the adherent cells remaining after removal of the nonadherent cells. The second band contained the heterophils, the chicken equivalent of the neutrophil. These cells were washed twice in PBS and were cultured for 2 h in Dulbecco minimal essential medium with 10% serum to remove contaminating erythrocytes. The pellet, which contained the erythrocytes, was washed twice in PBS before stimulation. The identity of the cells was confirmed by microscopic analysis of cells stained with Dif-Quick differential stain (Sigma Chemical Company).

RNA isolation and Northern (RNA) blot analysis. RNA was extracted by the guanidine hydrochloride method (15). Whole-cell RNA was denatured, electrophoresed through a 1.2% agarose–formaldehyde gel, transferred to a Zeta Probe nylon membrane (Bio-Rad Laboratories, Richmond, Calif.), and probed by standard methods (24). 9E3/CEF4 cDNA insert was labeled with [^{32}P]dCTP by the random priming

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method (13) with an oligolabeling kit (Pharmacia Inc., Piscataway, N.J.).

Purification of 9E3/CEF4 protein. The purification of 9E3/CEF4 protein from culture fluids of CEF will be described elsewhere (17a). Briefly, concentrated culture medium from RSV-transformed CEF was partially purified on a CM-Sepharose gel column (Pharmacia) and was further purified and concentrated to a single peak of 6 kDa on a Mono S HR 5/5 column (Pharmacia) by fast-protein liquid chromatography.

CEF preparation, culture, and infection. Primary cultures of CEF were prepared, maintained, and infected as previously described (18). Secondary cultures of CEF were infected with Schmidt-Ruppin RSV subgroup A (9) as previously described and were cultured in F-10 medium with 5% serum until they were transformed. Uninfected and transformed CEF were seeded at a density of 10^6 cells per ml in F-10 with 5% serum and were cultured overnight in F-10 with either no serum or 5% serum before the start of each experiment.

Thymidine incorporation. A total of 2×10^5 uninfected or transformed CEF were seeded in 24-well plates, in medium F-10 with 5% serum. The medium was removed after 4 to 8 h and replaced with F-10 containing no serum or 5% serum, and the cells were incubated for 16 h. The medium was then replaced with F-10 containing no serum, and the cells were labeled with 4 μCi of [^{3}H]thymidine (Du Pont, NEN Research Products) for 2 h. After the pulse, the cells were treated with 10% trichloroacetic acid, the precipitates were collected by filtration through glass fiber filters, and the radioactivity was measured by scintillation counting. The counts per minute were normalized to 2×10^5 cells.

Chemotaxis. Chemotaxis of peripheral leukocytes was measured by the Boyden double-membrane technique, with a 0.4-μm filter (Nuclepore) for the bottom filter and a 5.0-μm filter for the top filter. After chemotaxis had been allowed to take place at 37°C for 40 min, the medium was aspirated from the top chamber, and the bottom filter was fixed in methanol and the cells were stained with Dif-Quick differential stain (Sigma Chemical Company). Chemotaxis of CEF was measured in Boyden chambers, with a single 8.0 μm filter which had been precoated with gelatin (30, 31). After 1.5 h of incubation at 37°C, the medium was aspirated, and the cells were scraped from the top of the filter. After cells were fixed and stained as described above, the filter was inverted and the cells which had migrated to the bottom of the filter were counted. All chemotaxis samples were done in duplicate, and the cell counts on each filter were done three times.

Production of 9E3/CEF4 fusion protein and 9E3/CEF4 antiserum. A 390-bp *Pvu*II-*Sau*3A fragment derived from 9E3/CEF cDNA (44) was cloned into the fusion expression vector pJL6 (21). This vector contains a unique *Cla*I site 12 codons beyond the bacteriophage lambda cII initiation codon, allowing expression of the 9E3/CEF4 protein fused to the amino-terminal portion of the cII gene. Synthesis of the hybrid protein was induced in transformed *Escherichia coli* N4830-1 (Pharmacia) by heat shock at 42°C for 2 to 4 h. The protein was purified by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the gel bands containing the protein were injected subcutaneously into New Zealand White rabbits, and the immune serum was collected after the third boost.

Preparation of CEF ECM. Confluent cultures of uninfected and RSV-transformed CEF were washed with PBS with 5 mM EDTA and were then incubated in PBS with 5 mM EDTA for 5 min to detach the cells from the plates. The remaining cells were lysed and removed from the extracellular matrix (ECM) by a wash and two 10-min incubations with 0.025 M NH_4OH at room temperature. The ECM was washed twice in PBS, incubated for 10 min in 70% ethanol, and washed with PBS before the immunofluorescence assay was performed (12).

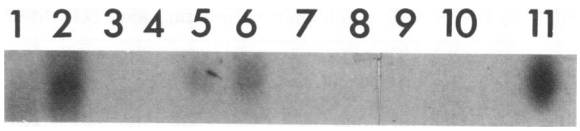

FIG. 1. 9E3/CEF4 mRNA expression in chicken peripheral blood cells. Monocytes, lymphocytes, erythrocytes, and heterophils were isolated from chicken peripheral blood as described in Materials and Methods. The cells were cultured in Dulbecco modified Eagle medium and were stimulated with lipopolysaccharide or with PMA for 30 min before RNA collection. After Northern analysis, the blot was probed with ^{32}P-labeled 9E3/CEF4 cDNA. Lane 1, monocytes; lane 2, monocytes stimulated with lipopolysaccharide; lane 3, heterophils; lane 4, heterophils stimulated with PMA; lane 5, monocytes; lane 6, monocytes stimulated with PMA; lane 7, lymphocytes; lane 8, lymphocytes stimulated with PMA; lane 9, erythrocytes; lane 10, erythrocytes stimulated with PMA; lane 11, CEF transformed by RSV.

Immunofluorescence. CEF and ECM were washed in PBS and were fixed in methanol at −20°C for 5 min and in acetone at −20°C for 2 min. The specimens were blocked with 5% goat serum in PBS for 30 min at room temperature, and antibodies were added at a concentration of 1:100 in PBS with 5% goat serum. The antibodies used were either normal rabbit serum or rabbit anti-9E3/CEF4, which was made against bacterially expressed 9E3/CEF4. After extensive washing with PBS, goat anti-rabbit immunoglobulin G-fluorescein isothiocyanate conjugate (Bethesda Research Laboratories) was added at a concentration of 1:100, and the specimens were incubated for 30 min at room temperature before observation and photography on a Zeiss immunofluorescence microscope.

RESULTS

Expression of 9E3/CEF4 mRNA in chicken peripheral blood cells. 9E3/CEF4 has been found to be expressed in CEF (5, 44) and in connective tissue, tendon, and bone (25, 26). We examined the expression of 9E3/CEF4 mRNA in chicken peripheral blood cells, to see whether its expression would be similar to that of IL-8 and related cytokines (Fig. 1). 9E3/CEF4 was expressed only in monocytes, and this expression was stimulated by the addition of phorbol myristic acetate (PMA) or lipopolysaccharide.

Several chicken tissues were examined by Northern blot for the expression of 9E3/CEF4 mRNA. No signal was seen in chicken muscle, cerebellum, intestine, spleen, telecephalon, or lung (data not shown). It is likely that stimulation of some cell types is necessary to cause 9E3/CEF4 expression, as is the case for untransformed CEF (44).

Chemotaxis of peripheral blood cells in response to 9E3/CEF4. Several cell types, including neutrophils, monocytes, and T cells, are known to respond to proteins of the PF4 family by migration towards the protein. We examined the ability of peripheral blood mononuclear cells (PBMC) and heterophils for chemotaxis in response to purified 9E3/CEF4 in a Boyden chamber assay (Table 1). Both the PBMC and the heterophils migrated across a filter toward 9E3/CEF4. The maximal response of PBMC to 9E3/CEF4 was seen at a

TABLE 1. Chemotaxis of chicken peripheral blood cells to 9E3[a]

Cell type	Chemoattractant (concn [ng/ml])	No. of cells/field	% of control
PBMC	Medium alone	7.0	100
	Fibronectin	10.6	151
	9E3/CEF4 (1,250)	17.2	246
	9E3/CEF4 (500)	32.0	457
	9E3/CEF4 (50)	25.0	357
	9E3/CEF4 (5)	16.0	229
	9E3/CEF4 (0.5)	5.0	71
Heterophil	Medium alone	4.0	100
	9E3 (500)	11.1	278

[a] PBMC and heterophils were isolated from the peripheral blood of an adult chicken by double-gradient centrifugation. The cells were resuspended in F-10 medium without serum and used for a Boyden double-membrane chemotaxis assay. The chemotaxis assay was performed for 40 min at 37°C. The filters were stained, and the cells which had migrated to the 0.4-μm filter were counted. The results of a typical experiment are shown.

TABLE 2. Chemotaxis of CEF in response to 9E3/CEF4[a]

Cell type	Chemoattractant	No. of cells/field	% of control
CEF	None	0.8	100
	Fibronectin (25 μg/ml)	21.0	2,625
	9E3/CEF4 (50 ng/ml)	26.0	3,250
RSV-CEF[b]	None	1.3	100
	Fibronectin (25 μg/ml)	22.0	1,692
	9E3/CEF4 (50 ng/ml)	11.3	869

[a] Chemotaxis was measured by a Boyden chamber single-membrane chemotaxis assay. After chemotaxis for 1.5 h at 37°C, the cells which had migrated through the filter were counted at 400× magnification after staining. Fibronectin was used as a positive control for chemotaxis.
[b] RSV-CEF, RSV-transformed CEF.

9E3/CEF4 concentration of 500 ng/ml. The observed decreased chemotactic response at higher 9E3/CEF4 concentrations has been described for other chemoattractants and hormones (4) and results from receptor downregulation. It was not possible to discern by morphology which types of PBMC were migrating toward 9E3. We were not able to elicit a positive nonspecific esterase, a monocyte marker, from any chicken peripheral blood cells, and so this assay could not be used to distinguish between monocytes and lymphocytes on the bottom chemotaxis filter. However, two pieces of evidence suggest that it is chicken monocytes which are the primary chemotactic responders to 9E3. Immunoblotting of filters after chemotaxis of PBMC with an antibody directed against the chicken T-cell receptor yielded no signal (data not shown). Furthermore, depletion of monocytes from a lymphocyte-monocyte fraction by two rounds of adherence resulted in a cell population which did not respond chemotactically to 9E3/CEF4 (data not shown).

The heterophil response was not as dramatic as was that of the PBMC, which is likely because of the limited cell numbers obtainable. However, the response was extremely reproducible and was the same for several 9E3/CEF4 preparations.

Chemotaxis of CEF in response to 9E3/CEF4. Fibroblasts are motile cells and are known to migrate towards substances such as platelet-derived growth factor (42) and fibronectin (30). Since 9E3/CEF4 appears to be an inflammatory mediator, and since fibroblasts must be called to the site of a wound, it was possible that 9E3/CEF4 could act as a chemoattractant for fibroblasts. To test this hypothesis, 9E3/CEF4 was added to fibroblasts in Boyden chambers to measure the number of fibroblasts which would be stimulated to migrate through a filter towards 9E3/CEF4 (Table 2). While few cells passed through the filter when no chemoattractant was added to the other side of the filter, many cells migrated towards either 9E3/CEF4 or fibronectin. Interestingly, though uninfected and RSV-transformed CEF responded equally well to fibronectin as a chemoattractant, uninfected CEF responded over twice as well as did RSV-transformed CEF to 9E3/CEF4.

Mitogenicity of CEF in response to 9E3/CEF4. Stimulation of mitogenicity has been reported to be a property of PF4-related gro/MGSA (33, 34) and for connective tissue activating peptide III (8). We examined the effect of purified 9E3/CEF4 on [^{3}H]thymidine uptake into CEF, to see whether cell growth was stimulated by 9E3. CEF were incubated with 9E3/CEF4 in the presence or absence of serum, and [^{3}H]thymidine uptake was measured after 16 h (Table 3). CEF incubated with 500 μg of 9E3/CEF4 per ml in the presence of serum showed very little stimulation of growth, and CEF incubated with 500 μg of 9E3/CEF4 per ml without serum showed a twofold stimulation of thymidine uptake. Neither transformed nor nontransformed CEF responded to a lower concentration of 9E3/CEF4.

Another property ascribed to members of the PF4 family is the ability to modulate arachidonic acid metabolism (10, 19, 37). Arachidonic acid release was not affected by the addition of 9E3/CEF4 to CEF, heterophils, lymphocytes, or monocytes (data not shown).

Localization of 9E3/CEF4 on cells and in the ECM. To examine the localization of the endogenous secreted 9E3/CEF4 protein, immunofluorescence studies with anti-9E3/CEF4 antibody were done. Incubation of uninfected and RSV-transformed CEF (Fig. 2A) demonstrated that 9E3/CEF4 was associated with both control and transformed cells, although more 9E3/CEF4 was present on the transformed CEF.

9E3/CEF4 can bind in vitro to heparin, probably because the pI of the 9E3/CEF4 protein is 8.9 (17a). Since several proteins that bind heparin in vitro are also known to bind in vivo to the ECM (14, 35), we examined the association of 9E3/CEF4 with the ECM of uninfected and transformed CEF (Fig. 2B). While very little 9E3/CEF4 was associated with the ECM of the uninfected cells, a strong immunofluorescence signal from RSV-transformed CEF was visible. Immunoprecipitation of cells, medium, and ECM demon-

TABLE 3. [^{3}H]thymidine incorporation into CEF in response to 9E3/CEF4[a]

Serum concn (%)	9E3 concn (μg/ml)	[^{3}H]thymidine uptake (% of control)
0	50	158
	500	195
5	50	91
	500	124

[a] Infected and control CEF were maintained for 20 h in medium containing no serum or 5% serum, with or without 9E3/CEF4 protein. The cells were pulsed for 2 h with 4 μCi of [^{3}H]thymidine in medium without serum. The trichloroacetic acid-precipitable material was collected and counted, and the results were expressed as a percentage of the counts per minute in uninfected and untreated cells. All assays were done in duplicate and were repeated at least three times. A representative experiment is shown.

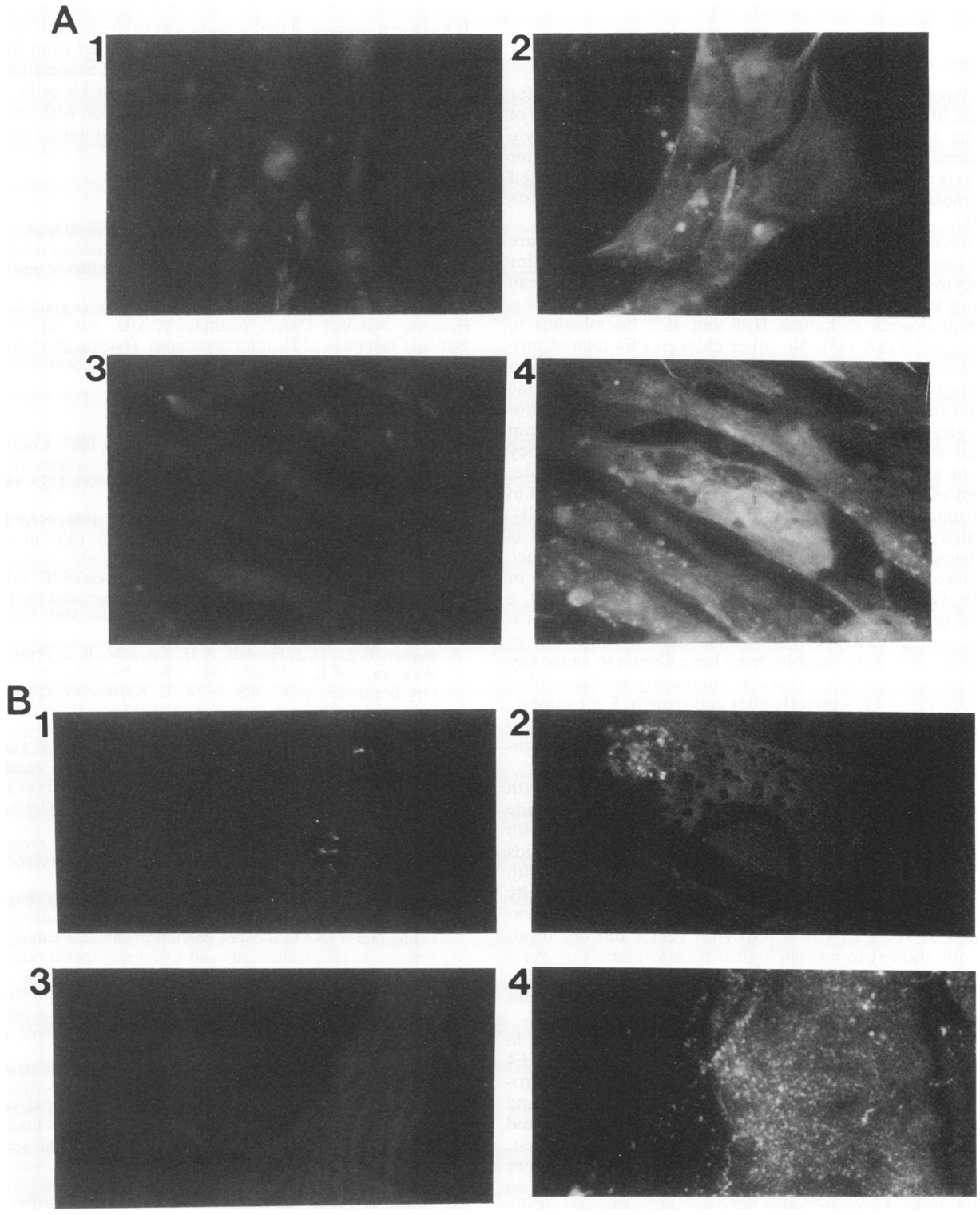

FIG. 2. Association of 9E3/CEF4 protein with CEF and with the ECM. (A) Uninfected (panels 1 and 2) and RSV-transformed CEF (panels 3 and 4) were fixed as described in Materials and Methods and were incubated with either nonimmune serum (panels 1 and 3) or anti-9E3 antibody (panels 2 and 4). Visualization was done with fluoresceinated anti-rabbit antibody. (B) Uninfected (panels 1 and 2) and RSV-transformed (panels 3 and 4) CEF were extracted with 0.25 M NH_4OH. The ECM remaining on the culture dish was fixed in the same way as were the CEF in panel A and was incubated with either nonimmune serum (panels 1 and 3) or anti-9E3 antibody (panels 2 and 4) before visualization with fluoresceinated anti-rabbit antibody.

strated that 9E3/CEF4 was precipitated from the same ECM fraction as was fibronectin (data not shown).

DISCUSSION

We have shown that 9E3/CEF4 mRNA is chemotactic for chicken PBMC and heterophils. Several of the PF4 family of proteins are also chemotactic for monocytes: monocyte chemotaxis protein-1/monocyte chemotaxis activating factor (47, 48) is a chemotactic factor for monocytes, and PF4 itself is chemotactic for monocytes, neutrophils, and fibroblasts (11, 41).

However, though both IL-8 (2) and gro/MGSA (40) are chemotactic for neutrophils, neither is chemotactic for monocytes. This makes it difficult to ascribe a human homolog to 9E3/CEF4. At the amino acid level, 9E3/CEF4 is 51% homologous to human IL-8 and 45% homologous to human gro/MGSA (43). No other chicken PF4-related protein has been described, and it could be that the chicken homolog of IL-8 is yet to be found. It is also possible that one chicken protein does the work of several mammalian proteins. gro/MGSA, with neutrophil activating peptide 2, can interact with the IL-8 receptor (29), and this redundancy could be more pronounced in the more primitive vertebrate.

Many of the members of the PF4 family were found first in transformed (1, 5, 44) or rapidly growing (17, 38) cells. Although the expression of the PF4 family of proteins is correlated with increased cell growth, there is little evidence that these proteins play an active role in the stimulation of growth. Only connective tissue activating peptide III (8), a platelet protein which acts as a mitogen for synovial fibroblasts, and gro/MGSA (33), which stimulates growth in melanoma cells only twofold, have been shown to cause any increased cell growth. There is increasing evidence that these proteins do not directly alter cell growth. For example, it has recently been demonstrated that KC, a murine *gro* homolog, can be downregulated without an effect on continued cell cycle progression (32).

9E3/CEF4, like gro/MGSA, stimulated autocrine growth twofold. There was a greater stimulation of [^{3}H]thymidine uptake in CEF incubated without serum than in that with serum. This could occur either because the 9E3/CEF4 binds to serum components and less is available to interact with the cells, or because cells growing in serum are maximally stimulated and 9E3/CEF4 is, therefore, ineffective. Overexpression of 9E3/CEF4 in a retroviral vector did not result (data not shown) in morphological transformation or overt increase in cell growth. These data suggest that 9E3/CEF4, as well as the other members of the PF4 family, does not play a major role in the stimulation of growth.

Considering the normal scenario of cell appearance in wound healing, it is interesting that the amount of 9E3/CEF4 needed to effect chemotaxis is 1,000-fold lower for heterophils than for fibroblasts. It may be that the neutrophil and the monocyte, which are early cells at the site of a wound (46), contain more 9E3/CEF4 receptors than the fibroblast, and so respond first; the release from monocytes of more 9E3, and of other chemotactic factors for fibroblasts such as platelet-derived growth factor (42), would finally recruit the fibroblast into the area.

9E3/CEF4 was found not only on the cell surface but also in the ECM. This has also been seen in vivo: in normal chicken tissues, 9E3/CEF4 is expressed in the cells and ECM of connective tissue (25). It is likely that 9E3, like the heparin-binding growth factors alpha fibroblast growth factor (FGF), basic FGF, int-2, k-FGF, and FGF-5 (6), and granulocyte macrophage-colony stimulating factor and IL-3 (36), associates with the ECM by binding to heparin sulfate. It has been suggested that attachment to the ECM may act as storage for a protein, in which it is protected from thermal denaturation or extremes of pH (16). ECM association may also facilitate presentation of that protein to cells. Since 9E3/CEF4 is a chemotactic factor, association with the ECM may provide a way of maintaining a chemotactic stimulus during inflammation.

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REFERENCES

1. **Anisowicz, A., L. Bardwell, and R. Sager.** 1987. Constitutive overexpression of a growth-related gene in transformed Chinese hamster and human cells. Proc. Natl. Acad. Sci. USA **84:**7188–7192.
2. **Baggiolini, M., A. Walz, and S. L. Kunkel.** 1989. NAP/IL-8, a novel cytokine that activates neutrophils. J. Clin. Invest. **84:** 1045–1049.
3. **Barker, K., and H. Hanafusa.** 1990. Expression of 9E3 mRNA is associated with mitogenicity, phosphorylation, and morphological alteration in chicken embryo fibroblasts. Mol. Cell. Biol. **10:**3813–3817.
4. **Becker, E. L., H. J. Showell, P. H. Naccache, R. J. Freer, R. W. Walenga, and R. I. Shaafi.** 1982. Chemotactic factors: locomotory hormones, p. 87–103. *In* M. L. Karnovsky and L. Bolis (ed.), Phagocytosis, past and future. Academic Press, Inc., New York.
5. **Bedard, P.-A., D. Alcorta, D. L. Simmons, K.-C. Luk, and R. L. Erikson.** 1987. Constitutive expression of a gene encoding a polypeptide homologous to biologically active human platelet protein in Rous sarcoma virus-transformed fibroblasts. Proc. Natl. Acad. Sci. USA **84:**6715–6719.
6. **Burrus, L. W., and B. B. Olwin.** 1989. Isolation of a receptor for acidic and basic fibroblast growth factor from embryonic chick. J. Biol. Chem. **264:**18647–18653.
7. **Carveth, H. J., J. F. Bohnsack, T. M. McIntyre, M. Baggiolini, S. M. Prescott, and G. A. Zimmerman.** 1989. Neutrophil activating factor (NAF) induces polymorphonuclear leukocyte adherence to endothelial cells and to subendothelial matrix proteins. Biochem. Biophys. Res. Commun. **162:**387–393.
8. **Castor, C. W., J. W. Miller, and D. A. Walz.** 1983. Structural and biological characteristics of connective tissue activating peptide (CTAP-III), a major human platelet-derived growth factor. Proc. Natl. Acad. Sci. USA **80:**765–769.
9. **Cross, F. R., and H. Hanafusa.** 1983. Local mutagenesis of Rous sarcoma virus: the major sites of tyrosine and serine phosphorylation are dispensable for transformation. Cell **34:**597–607.
10. **Dahinden, C. A., Y. Kurimoto, A. De Weck, I. Lindley, B. Dewald, and M. Baggiolini.** 1989. The neutrophil-activating peptide NAF/NAP-1 induces histamine and leukotriene release by interleukin 3-primed basophils. J. Exp. Med. **170:**1787–1792.
11. **Deuel, T. F., R. M. Senior, D. Chang, G. L. Griffin, R. L. Heinrikson, and E. T. Kaiser.** 1981. Platelet factor 4 is chemotactic for neutrophils and monocytes. Proc. Natl. Acad. Sci. USA **78:**4584–4587.
12. **Fairbairn, S., R. Gilbert, G. Ojakian, R. Schwimmer, and J. P. Quigley.** 1985. The extracellular matrix of normal chick embryo fibroblasts: its effect on transformed chick fibroblasts and its proteolytic degradation by the transformants. J. Cell Biol. **101:**1790–1798.

13. **Feinberg, A., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments of high specific activity: addendum. Anal. Biochem. **137:**266–267.
14. **Folkman, J., M. Klagsbrun, J. Sasse, M. Wadzinski, D. Inger, and I. Vlodavsky.** 1988. A heparin-binding angiogenic protein-basis fibroblast growth factor is stored within basement membrane. Am. J. Pathol. **130:**393–400.
15. **Ginsburg, D., R. I. Handlin, D. T. Bonthron, T. A. Donlon, G. A. P. Bruns, S. A. Latt, and S. H. Orkin.** 1985. Human von Willebrand factor: isolation of complementary DNA clones and chromosomal location. Science **228:**1401–1406.
16. **Gospodarowicz, D., and J. Cheng.** 1986. Heparin protects basic and acidic FGF from inactivation. J. Cell. Physiol. **128:**475–484.
17. **Gottlieb, A. B., A. D. Luster, D. N. Posnett, and D. M. Carter.** 1988. Detection of gamma interferon-induced protein IP-10 in psoriatic plaques. J. Exp. Med. **168:**941–948.
17a. **Hampe, A., and H. Hanafusa.** Unpublished data.
18. **Hanafusa, H.** 1969. Rapid transformation of cells by Rous sarcoma virus. Proc. Natl. Acad. Sci. USA **63:**318–325.
19. **Hope, W., T. J. Martin, C. N. Chesterman, and F. J. Morgan.** 1979. Human beta-thromboglobulin inhibits PGI2 production and binds to a specific site in bovine aortic endothelial cells. Nature (London) **282:**210–212.
20. **Larsen, C. G., A. O. Anderson, E. Appella, J. J. Oppenhein, and K. Matsushima.** 1989. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. Science **243:** 1464–1466.
21. **Lautenberger, J. A., D. Court, and T. S. Papas.** 1983. High level expression in Escherichia coli of the carboxy-terminal sequences of the avian myelocytomatosis virus (MC29) v-myc protein. Gene **23:**75–84.
22. **Lindley, I., H. Aschauer, J.-M. Seifert, C. Lam, W. Brunowsky, E. Kownatzi, M. Thelen, P. Peveri, B. Dewald, V. Von Tsharner, A. Walz, and M. Baggiolini.** 1988. Synthesis and expression in Escherichia coli of the gene encoding monocyte-derived neutrophil-activating factor: biological equivalence between natural and recombinant neutrophil-activating factor. Proc. Natl. Acad. Sci. USA **85:**9199–9203.
23. **Maione, T. E., G. S. Gray, J. Petro, A. J. Hunt, A. L. Donner, S. I. Bauer, H. F. Carson, and R. J. Sharpe.** 1990. Inhibition of angiogenesis by recombinant human platelet factor-4 and related peptides. Science **247:**77–79.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Martins-Green, M., A. Aotaki-Keen, L. M. Hjelmeland, and M. J. Bissell.** 1992. The 9E3 protein: immunolocalization in vivo and evidence for multiple forms in culture. J. Cell Sci. **101:**701–707.
26. **Martins-Green, M., and M. J. Bissell.** 1990. Localization of 9E3/CEF-4 in avian tissues: expression is absent in Rous sarcoma virus-induced tumors but is stimulated by injury. J. Cell Biol. **110:**581–595.
27. **Martins-Green, M., C. Tilley, R. Schwartz, C. Hatier, and M. J. Bissell.** 1992. Wound-factor-induced and cell cycle phase dependent expression of 9E3/CEF4, the avian gro gene. Cell Regul. **101:**701–707.
28. **Matsushima, K., K. Morishita, T. Yoshimura, S. Lavu, Y. Kobayashi, W. Lew, E. Apella, H. F. Kung, E. J. Leonard, and J. J. Oppenheim.** 1988. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J. Exp. Med. **167:**1883–1893.
29. **Moser, B., C. Schumacher, V. Von Tscharner, I. Clark-Lewis, and M. Baggiolini.** 1991. Neutrophil-activating peptide 2 and *gro*/melanoma growth-stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils. J. Biol. Chem. **266:**10666–10671.
30. **Postlethwaite, A. E., J. Keski-Oja, G. Balan, and A. H. Kang.** 1981. Induction of fibroblast chemotaxis by fibronectin. J. Exp. Med. **153:**494–499.
31. **Postlethwaite, A. E., R. Snyderman, and A. H. Kang.** 1976. The chemotactic attraction of human fibroblasts to a lymphocyte-derived factor. J. Exp. Med. **144:**1188–1203.
32. **Rameh, L. E., and M. C. S. Armelin.** 1992. Downregulation of JE and KC genes by glucocorticoids does not prevent the $G_0 \rightarrow G_1$ transition in BALB/3T3 cells. Mol. Cell. Biol. **12:**4612–4621.
33. **Richmond, A., E. Balentien, H. G. Thomas, G. Flaggs, D. E. Barton, J. Spiess, R. Bordoni, U. Francke, and R. Derynck.** 1988. Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structurally related to beta-thromboglobulin. EMBO J. **7:**2025–2033.
34. **Richmond, A., and H. G. Thomas.** 1988. Melanoma growth stimulatory activity: isolation from human tumors and characterization of tissue distribution. J. Cell. Biochem. **36:**185–198.
35. **Rifkin, D. B., and D. Moscatelli.** 1989. Recent developments in the cell biology of basic fibroblast growth factor. J. Cell Biol. **109:**1–6.
36. **Roberts, R., J. Gallagher, E. Spooncer, T. D. Allen, F. Bloomfield, and T. M. Dexter.** 1988. Heparin sulphate bound growth factors: a mechanism for stromal cell mediated haemopoiesis. Nature (London) **232:**376–378.
37. **Schroder, J.-M.** 1989. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates human neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. J. Exp. Med. **170:**847–863.
38. **Schröder, J.-M.** 1991. Biochemical and biological characterization of NAP-1/IL-8-related cytokines in lesional psoriatic scale. Adv. Exp. Med. Biol. **305:**97–107.
39. **Schroder, J.-M., N. L. M. Persoon, and E. Christophers.** 1990. Lipopolysaccharide-stimulated human monocytes secrete apart from NAP-1/IL-8, a second neutrophil-activating protein. J. Exp. Med. **171:**1091–1109.
40. **Schroder, J.-M., M. Stricherling, H. H. Henneicke, W. C. Preissner, and E. Christophers.** 1992. Il-1 alpha or tumor necrosis factor-alpha stimulate release of three NAP-1/IL-8-related neutrophil chemotactic proteins in human dermal fibroblasts. J. Immunol. **144:**2223–2232.
41. **Senior, R. M., G. L. Griffin, J. S. Huang, D. A. Walz, and T. F. Deuel.** 1983. Chemotactic activity of platelet alpha granule proteins for fibroblasts. J. Cell Biol. **96:**382–385.
42. **Seppa, H., G. Grotendorst, S. Seppa, E. Schiffman, and G. R. Martin.** 1982. Platelet-derived growth factor is chemotactic for fibroblasts. J. Cell Biol. **92:**584–588.
43. **Stoeckle, M. Y., and K. A. Barker.** 1990. Two burgeoning families of platelet factor 4-related proteins: mediators of inflammatory responses. New Biol. **2:**313–323.
44. **Sugano, S., M. Y. Stoeckle, and H. Hanafusa.** 1987. Transformation by Rous sarcoma virus induces a novel gene with homology to a mitogenic platelet protein. Cell **49:**321–328.
45. **Thelen, M., P. Reveri, P. Kernen, V. Von Tscharner, A. Walz, and M. Baggiolini.** 1988. Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. FASEB J. **2:**2702–2706.
46. **Wong, H. L., and S. M. Wahl.** 1989. Tissue repair and fibrosis, p. 383–394. *In* R. Ignotz and J. Massaque (ed.), Human monocytes. Academic Press, Inc., New York.
47. **Yoshimura, T., E. A. Robinson, S. Tanaka, E. Appella, J.-I. Kuratsu, and E. J. Leonard.** 1989. Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. J. Exp. Med. **169:**1449–1459.
48. **Yoshimura, T., N. Yuhki, S. K. Moore, E. Appella, M. I. Lerman, and E. J. Leonard.** 1989. Human monocyte chemoattractant protein-1 (MCP-1): full length cDNA cloning, expression in mitogen-stimulated blood mononuclear leukocytes, and sequence similarity to mouse competence gene JE. FEBS Lett. **244:**487–493.