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INDUCTION OF HAPTOTACTIC MIGRATION OF MELANOMA CELLS BY NEUTROPHIL ACTIVATING PROTEIN/INTERLEUKIN-8

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Natural or recombinant neutrophil activating cytokine (IL-8) induced migration across polycarbonate filters of human A 2058 melanoma cells. Anti-IL-8 antibodies blocked IL-8 induced melanoma cell migration. Checkerboard experiments revealed a gradient-dependent response of A2058 melanoma cells to IL-8. Filters exposed to IL-8 and washed supported melanoma cell migration, thus implying a haptotactic component in the response. The homologous polypeptide platelet factor 4 was inactive. The observation that IL-8 affects melanoma cells emphasizes the need for a comprehensive analysis of the spectrum of action of platelet factor 4-related peptides. The effect of the inflammatory cytokine IL-8 on melanoma cells may be relevant to augmented secondary localization of tumors at sites of inflammation.

Neutrophil activating protein/interleukin-8 (IL-8) is a recently identified cytokine which induces migration and activation of neutrophilic polymorphonuclear leukocytes (1-7). IL-8 is also active on basophils (8) and lymphocytes (9), but not on mononuclear phagocytes. Various cell types, including monocytes, endothelial cells and fibroblasts can produce IL-8. IL-8 belongs to an emerging group of cytokines involved in inflammation. These include platelet factor 4 (PF4; ref.10), macrophage inflammatory proteins (MIP1 and 2) (11, 12), a cytokine chemotactic for monocytes (13-15), a growth factor active on melanocytes (16, 17), as well as cDNA clones which encode products whose function is unknown. The structural hallmark of these mediators is represented by the conserved position of 4 cysteins. While it is clear that several of these cytokines are involved in the regulation of leukocyte recruitment and activation, the spectrum of action of individual members of

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this group of peptide mediators remains to a large extent to be defined. Here we report that IL-8 induces haptotactic migration of melanoma cells.

MATERIALS AND METHODS

Cells and chemoattractants

A2058 human melanoma cells (18) were maintained in culture in DME supplemented with 10% FCS. Cells in logarithmic phase of growth were detached by brief exposure to 0.05% trypsin/0.02% EDTA and kept in DME 10% FBS for 20 min before the assay. The cells were then washed twice with PBS and resuspended in DME 1%FBS at 1 X 10^6/ml. Recombinant human neutrophil activating factor (rhIL-8) was prepared as described (19). Human natural IL-8 was purified to homogeneity from supernatants of stimulated mononuclear cells as described (20). Specific neutralizing antibody against pure IL-8 was prepared in goat (21). Recombinant and natural IL-8 at the dilutions used were endotoxin free as measured by Limulus amebocyte lysate assay. A 2058 melanoma cell conditioned medium defined autocrine motility factor (AMF; ref.22) was used in the migration assays as a reference positive control. Platelet factor 4 (PF4) purified to homogineity was a kind gift of Dr. M. Prosdocimi, fidia, Abano, Italy. Its biological activity was assessed as inhibition of heparin anti-coagolation.

Migration assays

Chemotaxis was assayed by a microchamber technique (23). 25 µl of stimuli were placed in the lower compartment of the chamber and 50 µl of A2058 melanoma cell suspension were seeded in the upper compartment. The two compartments were separated by a 8 µm pore-size polyvinylpyrrolidone (PVP)-free polycarbonate filter (Nuclepore Corp., Pleasantan, CA) coated with 100 µg/ml gelatin solution (Sigma Chemical, ST. Louis, MO, USA) as described (24). We subsequently found that uncoated filters gave similar migration of A 2058 cells to IL-8. The chamber was incubated at 37°C for 4 h. At the end of the incubation, filters were removed, fixed and stained with Diff-Quik (Harleco, Gibbstown, NJ), and 5 oil immersion fields were counted after coding the samples.

Haptotaxis was assessed using modified Boyden chambers (18). Dried polycarbonate filters (PVP-free, 13 mm diameter, 8 µm pore-size, Nuclepore) were coated on one side or both sides with rh IL-8 by floating for 4-20 h at 37°C on a solution of rh IL-8 (20 ng/ml). The filters (2 or 3 replicates /group) were washed once with assay medium and mounted in Boyden chambers. 200 ml medium were seeded in the lower compartment and 400 µl cell suspension were seeded in the upper. After 4 h incubation at 37°C, the filters were removed, stained and migrated cells were counted as for chemotaxis assay.

The significance of difference between numbers of migrated cells was assessed by Dunnet's test.

RESULTS

Table 1 shows two experiments representative of 10 performed, in which we examined the effect of IL-8 on the migration of melanoma cells across gelatin-coated polycarbonate filters. IL-8, seeded in the lower compartment of chemotaxis chambers, induced migration of A 2058 melanoma cells. A significant response was usually observed at a concentration of 1 ng/ml and maximal

Table 1. Induction of A2058 melanoma cell migration by IL-8[a]

Chemoattractant	Number of migrated cells (Mean ± SD)	
	Exp. 1	Exp. 2
Medium	83 ± 6	107 ± 9
AMF	106 ± 7[b]	184 ± 9[b]
rh IL-8 100ng/ml	130 ± 6[b]	159 ± 6[b]
10ng/ml	136 ± 14[b]	155 ± 6[b]
5ng/ml	NT	131 ± 4[b]
1ng/ml	122 ± 14[b]	116 ± 9
0.1ng/ml	76 ± 8	NT
rh IL-8 10ng/ml+anti-IL-8	NT	114 ± 14[c]
rh IL-8 10ng/ml+normal goat serum	NT	161 ± 1
Medium	82 ± 10	130 ± 7
AMF	122 ± 17[b]	240 ± 8[b]
Natural IL-8 10U/ml	104 ± 8 [b]	188 ± 9[b]
1U/ml	134 ± 11[b]	220 ± 18[b]
0.1U/ml	112 ± 6[b]	168 ± 13[b]
0.01U/ml	93 ± 10	NT
Natural IL-8 1U/ml + anti-IL-8	78 ± 7[c]	124 ± 9[c]

[a] Different dilutions of rh IL-8 or natural IL-8 were seeded in the lower compartments of the micro-chemotaxis chamber. Results are numbers of migrated A2058 melanoma cells in 5 oil fields with three replicates. Experiments are representative of 10 done for both types of IL-8. Goat anti-IL-8 antiserum was used at a concentration of 1:50.

[b] $p<0.05$ vs migration to medium control.

[c] $p<0.05$ vs migration to the same concentration of IL-8 without antibody.

migration was detected at 10-100 ng/ml. Thus the responsiveness of A 2058 melanoma cells to IL-8 is comparable to that of PMN. Highly purified natural IL-8 was also able to induce melanoma cell migration (Table 1). That IL-8 was indeed responsible for induction of melanoma cell migration was demonstrated by blocking with specific anti-IL-8 antibodies (Table 1). The structurally related protein PF4 and the leukocyte chemoattractant FMLP were inactive (data not shown, see also below).

In an effort to elucidate whether migration into filters of melanoma cells in response to IL-8 depended on the presence of a concentration gradient between the lower and upper compartments of the chamber, we performed a checkerboard experiment seeding different concentrations of the agent above and below the filter. As shown in Table 2, maximal induction occurred in the presence of a positive concentration gradient between the lower and upper compartments. However, also in the absence of a positive gradient, some enhanced migration was observed.

Signals that induce migration of adherent cells can be "seen" by cells either as soluble molecules or as substrate-bound molecules (haptotaxis, ref.18). To investigate this latter possibility, filters were exposed to IL-8 and washed; their capacity to support migration of melanoma cells was then examined. As shown in Table 3, where 2 experiments representative of 4

Table 2. Checkerboard analysis of A2058 melanoma cell migration across polycarbonate filters induced by IL-8[a]

			Above the filter IL-8 concentration	
Below	Medium	0.1U/ml	0.5U/ml	1U/ml
Medium	86 ± 6	101 ± 5	109 ± 1	90 ± 11
IL-8				
0.1U/ml	102 ± 1	104 ± 6	92 ± 7	97 ± 4
0.5U/ml	131 ± 8[b]	127 ± 8[b]	100 ± 1	106 ± 15
1U/ml	132 ± 10[b]	126 ± 5[b]	120 ± 5[b]	119 ± 10[b]

[a] Different concentrations of natural IL-8 were seeded in the upper and/or lower compartments of the micro-chemotaxis chamber. Results a number of migrated cells (Mean + SD) in 5 oil fields with three replicates.

[b] $P<0.05$ vs migration to medium control (above and below the filter).

performed are shown, filters exposed to IL-8 for 20 h and washed caused migration of melanoma cells, thus demonstrating a haptotactic response to this cytokine. When the filters were coated for 4h, a significant haptotactic response was observed, though lower than when free IL-8 was seeded in the lower compartment (data not shown). Albumin coating had no effect on melanoma cell migration. PF4, a heparin binding polypeptide structurally homologous to IL-8, was inactive, both when directly seeded in the chamber or when used to coat the filters.

Table 3. rhIL-8-induced haptotaxis of A2058 melanoma cells[a]

Chemoattractant	Number of migrated cells
Exp. 1.	
Medium	86 (77, 95)
AMF	163 (162, 163)
Soluble IL-8	149 (142, 156)
IL-8 on the lower surface	140 (137, 143)
IL-8 on the upper surface	79 (72, 85)
IL-8 on both sides	108 (103, 112)
Exp. 2.	
Medium	187 ± 18
AMF	267 ± 23
IL-8 on the lower surface	236 ± 18
PF4 on the lower surface	173 ± 20

[a] Dried gelatin-coated polycarbonate filters were coated on one or both sides with rh IL-8 by floating for 20 h at 37°C on a solution of rh IL-8 (20 ng/ml). The filters (2 replicates /group and 3 replicates/group for Exp. 1 and 2 respectively) were washed once with medium and mounted in Boyden chambers. Results of one out of 4 experiments performed are presented as mean number of migrated cells in 5 oil fields with individual values in parenthesis (Exp. 1) or with SD (Exp. 2).

DISCUSSION

The results presented here show that IL-8, a cytokine identified as a neutrophil chemoattractant, induces haptotactic migration of A 2058 melanoma cells. In addition to neutrophils, IL-8 has been shown to affect T lymphocytes and basophils (8, 9), while it has no effect on monocytes, endothelial cells and NK cells (data not shown). The results presented here are the first demonstration of an action of this cytokine on non-hematopoietic elements.

IL-8 belongs to an emerging family of polypeptides, the prototype of which is PF4 (10). A member of this family, MGSA/gro, was identified also as a melanocyte growth factor (16, 17). IL-8 and MGSA/gro have 44% aminoacid sequence identity (3). The observation that IL-8 affects melanoma cells emphasizes the need for a comprehensive analysis of the biological spectrum of action of PF4-related polypeptides.

The observation of induction of melanoma cell haptotaxis by IL-8 may have in vivo relevance. Diverse inflammatory signals including IL-1 and TNF, induce IL-8 production in leukocytes, vascular cells and connective tissue cells. Augmented secondary localization of cancer has been documented at sites of inflammation (25) and in vivo inoculation of IL-1 augments metastasis of human melanoma cells in nude mice (26). Since IL-1 is a potent inducer of IL-8, local production of the latter cytokine could contribute to enhanced secondary inplantation of melanoma cells.

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