

Expression of Eosinophil Chemotactic Factors in Stomach Cancer

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Abstract

We have occasionally experienced eosinophilic abscess of the liver in patients with gastric carcinoma, suggesting that some eosinophil mobilizing (chemotactic and proliferative) factors might be produced by carcinoma cells. The aim of this study was to determine whether or not gastric carcinoma expresses the well-known eosinophil chemotactic factors (ECFs) and whether or not the expression is related to the histologic subtypes. Seventeen consecutive surgically removed tumor-bearing stomachs were collected: 7 signet ring cell type, 7 poorly differentiated tubular adenocarcinoma, and 3 moderately differentiated tubular adenocarcinoma. Hematoxylin-eosin stained sections were re-evaluated for eosinophil and mast cell infiltration. The expressions of IL-2, IL-5 and granulocyte-macrophage colony stimulating factor (GM-CSF) were examined by immunocytochemical stain. There was no available frozen tissue for IL-2 and IL-5 in one case. Gastric carcinoma expressed IL-2 in all 16 cases, IL-5 in 12 of 16 cases and GM-CSF in 10 of 17 cases. Of particular interest, 7 of 10 GM-CSF-expressing carcinomas were signet ring cell type. Even in the remaining 3 cases, most GM-CSF-positive cells were signet ring cells scattered within tubular adenocarcinoma. No correlation of ECF expression between either eosinophil/mast cell infiltration or peripheral blood eosinophilia was identified. In conclusion, most gastric carcinomas express the well-known ECFs and the expression of GM-CSF is specific for signet ring carcinoma cells.

Key Words: Gastric cancer, eosinophil chemotactic factor, GM-CSF, IL-2, IL-5, signet ring cell, eosinophil

INTRODUCTION

The association of malignant tumors and peripheral blood eosinophilia or tissue eosinophil infiltration has been uncommonly described.¹⁻⁶ A few reports have described gastric cancer accompanied by marked tissue eosinophil infiltrates and peripheral eosinophilia.^{7,8} Gastric cancer accompanied by eosinophilic liver abscess was also described even in the absence of gastric tissue eosinophilia.⁹

The eosinophil infiltration in and around the tumor has been reported to suggest a poor prognosis in histiocytic lymphoma and uterine cervical carcinoma,³ whereas a favorable prognosis in gastric carcinoma.⁸ Regardless of the prognostic implication in gastric carcinoma, the eosinophil infiltration may produce

secondary achalasia of the esophagus without tumor infiltration.¹⁰ Furthermore we have not infrequently experienced massive hepatic infiltrations of eosinophil in patients with gastric carcinoma, suggesting that some eosinophil mobilizing (chemotactic and/or proliferative) factors may be produced by carcinoma cells.⁹ The first case report speculated that certain eosinophil mobilizing factor must be produced by signet ring cells.⁷ Another study showed that gastric carcinoma cell extract was highly chemotactic for eosinophils in vitro.⁸

The cytokines IL-2, IL-5, and GM-CSF, which are present in the blood and tissue of allergic individuals, modulate the transendothelial migration of eosinophils as well as the chemotactic responsiveness of eosinophils to various mediators and cytokines.¹¹ Eosinophils respond to T lymphocyte-derived cytokines, including LCF (lymphocyte chemoattractant factor) and IL-2.^{12,13} Since both LCF and IL-2 are potent chemoattractants, eosinophils could be recruited along with mononuclear inflammatory cells by these lymphokines that stimulate CD4-bearing and IL-2 receptor-expressing lymphocytes, respectively. Non-gastric tumors with eosinophilia, including lymphoma, leukemia, and carcinomas of the lung, uterine cervix and breast, have revealed these eosinophil chemotactic factors (ECFs)

Received December 14, 1998

Accepted February 26, 1999

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This study was supported by a faculty research grant of Yonsei University College of Medicine for 1996.

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in tumor cells.¹⁴⁻²³ But there has been no report about cytokine ECF expression in gastric cancer cells.

This study was therefore designed to determine whether gastric carcinoma expresses the well-known ECFs and to clarify the clinicopathologic significance of ECF expression in gastric carcinoma.

MATERIALS AND METHODS

Patients

Seventeen consecutive cases of gastric carcinoma collected during 1996 and 1997, in which adequate frozen section, paraffin blocks and clinical history were available, were retrieved from the files of the Department of Pathology, Yonsei University Wonju College of Medicine. By WHO classification,²⁴ 7 cases were signet ring cell carcinomas, 7 poorly differentiated tubular adenocarcinomas and 3 moderately differentiated tubular adenocarcinomas.

Histologic examination of eosinophil and mast-cell infiltration

Tissues were fixed in 10% neutral formalin, embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin. The Ziel-Neelsen stain for mast cell count was also performed.

Eosinophil and mast cell infiltration around the tumor cells in the mucosal layer and non-mucosal layers containing submucosa, muscle and serosa were counted separately. For eosinophil count, 10 consecutive 400x fields were examined and the mean values per field were calculated. Because eosinophil counts referenced from the literature have a normal limit of 20 per 400x field,²⁵ cases with more than 20 eosinophils per field were defined as positive. Mast cells are normally present in mucosa although few in numbers, but not in the muscle layer. Then we defined, according to a preliminary count, cases with more than 40 mast cells per 10 high power (400x) fields as positive.

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue sections (5 μ m thick) were heated at 60°C for 1 hour. The slides were then deparaffinized and hydrated through xylene and graded alcohols, and placed in distilled water. To block endogenous peroxidase acti-

vity, they were incubated for 10 minutes in 3% H₂O₂. The sections were microwaved (750 W) for 10 minutes in sodium citrate buffer (pH 6.0, 0.01 M) after washing in distilled water. After cooling for 20 minutes, they were incubated for 20 minutes in normal human serum (1 : 10 dilution) followed by overnight incubation in a refrigerator with polyclonal rabbit antihuman GM-CSF (Genzyme, Cambridge, MA, U.S.A. 1 : 10 dilution).

Frozen tissue sections (5 μ m thick) were air dried for 30 minutes. After the slides were fixed in cold acetone at 4°C for 10 minutes, they were air dried for 10 minutes. After the slides were incubated in Tris HCl buffer for 30 minutes, they were incubated for 20 minutes in normal human serum (1 : 10 dilution) followed by overnight incubation in a refrigerator with monoclonal mouse anti-human IL-2 (Biosource Int., Camarillo, CA, U.S.A. 1 : 50 dilution) and monoclonal rat anti-mouse IL-5 (Genzyme, Cambridge, MA, U.S.A. 1 : 50 dilution).

All sections were then rinsed and incubated for 30 minutes with biotinylated anti-mouse immunoglobulin and streptavidin-conjugated peroxidase (LSAB kit, Dako Corporation, Carpinteria, CA, U.S.A.). The peroxidase reaction was developed with 3-amino-9-ethylcarbazine. Negative controls were carried out by substituting primary antibody with mouse non-immune serum.

The ECF reaction was graded as 3+, 2+, 1+, and 0, according to the percentage of tumor cells showing positive cytoplasmic staining; over 50%, 10 to 50%, 1-10% and completely negative, respectively.

Statistical analysis

Statistically significant differences between the groups were determined using Fisher's exact test by SAS program.

RESULTS

ECF expression in gastric cancer cells

In 16 cases, intense cytoplasmic expression of IL-2 was demonstrated in gastric cancer cells. The expression was graded as 2+ (Fig. 1A) and nearly the same in all cases. IL-5 was also noted in 12 out of 16 cases. The expression of IL-5 was of weaker intensity than that of IL-2, and was graded as 1+. The lympho-

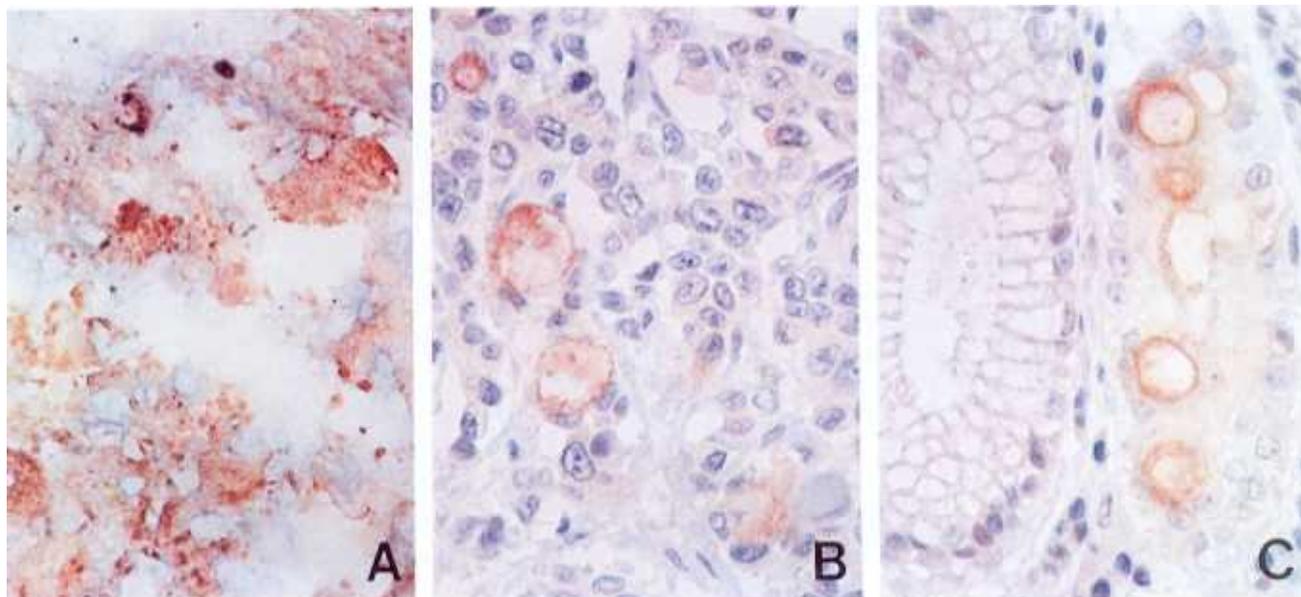


Fig. 1. Microphotograph of the immunohistochemical stains for eosinophil chemotactic factors. Gastric cancer cells in a frozen section showing strong IL-2 expression (A). Expression of GM-CSF in scattered signet ring cells of a poorly differentiated adenocarcinoma (B) and in metaplastic goblet cells of a non-neoplastic gastric tissue (C).

Table 1. Expression of Eosinophil Chemotactic Factors within Cancer Cells

No. of cases	GM-CSF † (n=17)	IL-2 (n=16)	IL-5 (n=16)
1	3+	2+	1+
2	2+	2+	1+
3	2+	2+	1+
4	1+	2+	1+
5	—	2+	1+
6	2+	2+	1+
7	—	2+	1+
8	—	1+	—
9	—	2+	1+
10	3+	2+	1+
11	—	2+	1+
12	3+	no frozen tissue	—
13	2+	2+	—
14	—	2+	—
15	1+	2+	1+
16	—	2+	—
17	3+	2+	1+
Total No. of positive cases	10	16	12

Reactions were graded as — (0), 1+ (1-10%), 2+ (10-50%) and 3+ (>50%).

Fisher's exact test $p > 0.01$.

† GM-CSF was also expressed in metaplastic goblet cells of all 17 cases in the grade of 1+.

cytes infiltrated around the tumor cells expressed neither IL-2 nor IL-5.

Ten out of 17 cases showed a relatively high-grade expression of GM-CSF in gastric cancer cells (Fig. 1B). Metaplastic goblet cells also showed high grade expression (Fig. 1C). The expression grades were somewhat variable; 1+ in two cases, 2+ in 4 cases, and 3+ in 4 cases (Table 1).

Relationship between the expression of eosinophil chemotactic factors and the infiltration of eosinophils and mast cells

Among 16 cases in which frozen sections and immunohistochemical study for IL-2 and IL-5 were available, 5 cases had peripheral blood eosinophilia which ranged from 5.1% to 10.0%. Of these 5 cases, GM-CSF was expressed in only 2 cases (Table 2), whereas IL-2 in all and IL-5 in 4 cases. Of the 11 cases without peripheral eosinophilia, IL-2 expression was found in all cases and IL-5 in 8 cases (data not shown).

Tissue eosinophil infiltration was noted in only 4 out of 17 cases and in the mucosal layer around the tumor cells. Two of these cases showed GM-CSF expression. Mast cell infiltration was found in only 5 cases, exclusively in the non-mucosal layers. Three of these 5 cases expressed GM-CSF. However, there was no statistical significance (Table 2).

Table 2. Relationship between Expression of GM-CSF and Tissue Infiltration of Eosinophils and Mast Cells

GM-CSF expression	Peripheral eosinophilia	Eosinophil infiltration*	Mast cell infiltration†
Positive (n=10)	2 (20.0%)	2 (20.0%)	3 (30.0%)
Negative (n=7)	3 (42.8%)	2 (28.5%)	2 (28.5%)
Total (n=17)	5	4	5

* Eosinophil infiltration in mucosa of stomach.

† Mast cell infiltration in non-mucosal layers of stomach.
Fisher's exact test $p > 0.01$.

Table 3. Relationship Between Eosinophil Chemotactic Factor Expression and Histologic Subtype of Gastric Carcinoma

Histologic subtype by WHO classification	Expression of ECF		
	IL-2	IL-5	GM-CSF
Signet ring cell carcinoma (n=7)	7 †	7 †	7 †
Tubular adenocarcinoma			
Poorly differentiated (n=7)*	6	3	2 §
Moderately differentiated (n=3)	3	2	1 §
Total	16/16	12/16	10/17

* No available frozen tissue for IL-2 and IL-5 in one of seven cases.

† Fisher's exact test $p > 0.01$.

‡ Fisher's exact test $p < 0.01$.

§ Positive cells were signet ring cells scattered within adenocarcinoma.

Relationship between ECF expression and histologic subtypes of carcinoma

Carcinoma of the signet ring cell type expressed all kinds of ECF without exception. Among 7 cases of poorly-differentiated tubular adenocarcinoma, IL-2 expression was seen in 6 cases, IL-5 in 3 cases and GM-CSF in 2 cases. In moderately-differentiated tubular adenocarcinoma, IL-2 was expressed in all 3, IL-5 in 2 and GM-CSF in 1 out of 3 cases. The GM-CSF expression in signet ring cell carcinoma proved to be statistically significant (Table 3). Even in 3 cases of the poorly and moderately differentiated tubular adenocarcinomas, most GM-CSF positive cells were signet ring cells scattered within tubular adenocarcinoma. Because of the frozen artifact, the cell type of IL-2 and IL-5 positive cells in the tubular adenocarcinoma could not be evaluated.

DISCUSSION

The present study demonstrates that gastric carcinomas are capable of expressing the ECFs; IL-2, IL-5 and GM-CSF. IL-2 was expressed in 100%, IL-5 in 75% and GM-CSF in 58.5% of the gastric carcinoma cases.

Previous studies have already revealed the expression of ECFs in non-gastric tumors and these studies suggested that the ECFs may be derived from two pathways; First, from the non-neoplastic lymphocytes and second, from the neoplastic cells themselves.^{14,16-22}

The first theory is supported by Hirashima et al.¹⁸ who showed that B cell lymphoma cells stimulate non-neoplastic T lymphocytes to secrete IL-2, and by Kita et al.²⁰ who revealed that nonleukemic T lymphocytes produce GM-CSF in an acute lymphoblastic leukemia.

The second theory is supported by the papers which reported that lung cancer cells,¹⁴ brain histiocytic lymphoma cells¹⁶ and uterine cervical carcinoma cells¹⁷ expressed ECF-A, ECF-HL and ECF-CC, respectively. They demonstrated the eosinophil chemotactic effects of tumor cells, but could not elucidate the exact nature of these ECFs. After 1990, several cytokines, such as GM-CSF, IL-3 and IL-2-like material, were reported to have eosinophilic chemotactic activity. It has been also demonstrated that cultured ATL cells secreted GM-CSF,¹⁹ myeloma cells expressed IL-3²² and BALL-1 tumor cells expressed IL-2-like material.²¹ Iwasaki et al.⁸ reported that the stomach cancer tissue extract expressed ECF, although they were unable to determine the source of ECF.

Taking into account the results of our study and previous reports, we can assert that tumor cells are capable of expressing ECF. The most interesting finding in our study was that all signet ring cell carcinomas expressed GM-CSF. Seven of 10 GM-CSF-expressing carcinomas were of signet ring cell type. And even in the remaining 3 cases, the large majority of GM-CSF positive cells were signet ring cells scattered within adenocarcinoma. In other previous reports, signet ring cells were found to express several kinds of molecules; IL-6, TGF-A, EGF receptor, CA125, CA19-9, cadherin-11, CEA, ICAM-I, and Lea antigen. The first 3 molecules, IL-6, TGF-A, and EGF receptor are associated with tumor growth.^{26,27} The remaining 5 molecules, CA125, CA19-9, cadherin-11, CEA and ICAM-I are related to tumor metastasis and aggressiveness.²⁷⁻³⁰ The Lea antigen is responsible for hemolytic anemia due to complement

consumption.³¹ However, there has been as yet no report on cytokine ECF expression by signet ring cells.

Our study could not determine whether GM-CSF expressions correlated with eosinophil/mast cell infiltration and peripheral blood eosinophilia. Only two of 10 cases expressing GM-CSF had peripheral blood eosinophilia. Very few cases showed eosinophil/mast cell infiltration around the tumor cells. So there was no statistical significance. Generally, the cytokine ECFs IL-3, IL-5, and GM-CSF present in the blood and tissue of allergic individuals modulate the transendothelial migration capacity of eosinophils, as well as the chemotactic responsiveness to various mediators and cytokines.¹¹ Eosinophils respond to T lymphocyte-derived cytokines, including LCF (lymphocyte chemoattractant factor) and IL-2.^{12,13} Since both LCF and IL-2 are potent eosinophil chemoattractants, eosinophils could be recruited along with mononuclear cells by these lymphokines which stimulate CD4-bearing and IL-2 receptor-expressing lymphocytes. Thereby, several tumors which can express these ECFs showed tissue and peripheral-blood eosinophilia.^{14,16-23} The above mechanisms are thought to be responsible for causing tissue and peripheral blood eosinophilia in ECF-expressing tumors. However, in our study, the GM-CSF-expressing cases were not related with eosinophilia. We propose two possibilities for this controversial finding. First, that ECF may cross-react with other molecules, and second, that the level of ECF in these case is too low for inducing eosinophilia.

As an incidental finding of our study, we discovered that goblet cells also expressed GM-CSF. According to a study by Kang and Kim³² on signet-cell morphological changes; type A cell is an immature small cell, but type C cell is similar to goblet cells in shape and mucus content. Since both signet ring cell carcinoma cells and metaplastic goblet cells expressed ECF in our study, we could assume that either the mucus itself contains GM-CSF or that it cross-reacts with GM-CSF. We know that normal gastric mucosa is generally infiltrated with a few eosinophils. So we may then assume that the GM-CSF expressing metaplastic goblet cells partly induces the infiltration of these eosinophils. Although some reports could not find a definite correlation between the appearance of metaplastic goblet cells and signet ring cell carcinoma,^{33,34} a recent report succeeded in demonstrating a relationship between signet ring cell carcinoma and goblet cells by using specific antibody to intestinal goblet cells.³⁵ Thus, the possible existence of a goblet cell metaplasia to signet ring cell carcinoma

sequence was also suspected. Our study has been limited by the small number of cases and the sensitivity of the detection methods. The above uncertainties, however, are likely to be solved by further studies with a larger number of cases.

In summary, gastric carcinomas can express ECFs and the expression of GM-CSF is specific for signet ring carcinoma cells. GM-CSF expression is not correlated with eosinophil infiltration. The role of GM-CSF in signet ring cells are expected to be ascertained by further studies.

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