Expression of the Chemokine Receptor CXCR2 in Normal and Neoplastic Neuroendocrine Cells

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• ***Background.*—Chemokines effect their proinflammatory and growth regulatory roles through interaction with serpentine receptors. One such receptor, CXCR2, binds multiple CXC chemokines, including interleukin 8, GRO-α, GRO-β, GRO-γ, and NAP-2. We have previously identified CXCR2 expression on myeloid cells, notably mature granulocytes, and projection neurons.**

***Objective.*—To determine the expression of CXCR2 by cells of the neuroendocrine system.**

***Design.*—Archival specimens from normal neuroendocrine tissues and their malignant counterparts were analyzed by immunohistochemistry with monoclonal antibodies specific for CXCR1 and CXCR2.**

***Results.*—Immunohistochemical analysis revealed high-level expression of CXCR2 by cells in the pituitary, adrenal medulla, pancreatic islets, thyroid C cells, scattered Kulchitsky cells in the bronchi, and counterpart neuroendocrine cells in the stomach, small bowel, colon, and appendix. Neuroendocrine neoplasms that demonstrated high-level CXCR2 expression included (1) primary carcinoids localized to the stomach, small bowel, colon, appendix, fallopian tube, ovary, and lung; (2) atypical carcinoids of the lung; (3) metastatic carcinoids; (4) pituitary adenomas; (5) pheochromocytomas; and (6) medullary carcinomas of the thyroid. Small cell lung carcinomas, large cell neuroendocrine carcinomas of the lung, small cell carcinoma of the cervix, Merkel cell carcinomas, neuroblastomas, and malignant melanomas lacked evidence of CXCR2 expression.**

***Conclusions.*—The expression of CXCR2 by normal neuroendocrine cells and neoplastic counterparts that have retained phenotypic features of this differentiation program suggests that chemokines may play an important role in functions that are characteristic of this cell type. In addition, this raises the possibility that chemokines may modulate secretion of biologically active products of these cells and their neoplastic counterparts.**

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The dispersed neuroendocrine system (DNS) is composed of cells in the central and peripheral nervous systems that subserve endocrine and paracrine functions.[1] They have been designated as paraneurons based on the common function of secreting biologically active agents, including proteins, polypeptides, and other compounds.[2] The targets of these products may be local or distant, thus defining paracrine and endocrine roles, respectively. Thus, these cells can be considered to be paraneurons, since their secretion products may act as neurotransmitters, which offers a broader perspective than the previous amine precursor uptake and decarboxylation designation.[3]

The normal elements of the DNS include cells in the adrenal medulla, biliary tract, liver, respiratory system, gastrointestinal tract, pituitary, epidermis (melanocytes and Merkel cells), pancreatic islets, parathyroid, thyroid (C cells), and cells dispersed in the breast, uterine cervix, kidney, larynx, ovary, paranasal sinuses, prostate, and testis. The neoplastic counterparts of these populations include neural tumors, such as pheochromocytomas and paragangliomas, and epithelial tumors that arise in various tissues, such as carcinoids, medullary carcinoma of the thyroid, pituitary adenomas, and other neuroendocrine adenomas and carcinomas.[4] Most of these neoplasms are functional and express and secrete the product corresponding to their terminal differentiation. In addition, there are several markers that are broadly expressed by DNS cells. These include neuron-specific enolase, chromogranin/secretogranin, synaptophysin, bombesin/gastrin-releasing peptide, and neural cell adhesion molecule (CD56).[5–13]

Chemokines are products of the largest cytokine gene family.[14] They promote inflammation by inducing the directed migration of leukocytes.[15,16] Additional roles include the modulation (both positive and negative) of angiogenesis[17,18] and growth regulatory functions.[19–21] The latter role was first described for the chemokine known as GRO-α, which was also discovered as an autocrine growth factor for melanomas.[19] Subsequent reports have shown that this chemokine is expressed at high levels in inflammatory disorders of the epidermis.[22] Receptors for chemokines are members of the serpentine receptor superfamily, which have 7 hydrophobic helices and are coupled to signal transduction pathways through heterotrimeric G proteins.[14] The receptor for GRO-α, designated CXCR2, has been described in epidermal cells in regions that cor-

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respond to intermediate levels of differentiation.[23,24] Its expression was further studied in a panel of nonhematopoietic tissues to gain insight into other functions of this receptor. Immunohistochemical analysis of CXCR2 expression revealed that it was universally expressed by cells in the DNS, in addition to squamous epithelial cells. Although the precise role for signaling through CXCR2 in the physiology of DNS cells has not been determined, it can serve as a differentiation marker for neoplasms derived from this cell type.

MATERIALS AND METHODS

All cases were selected from the files of the Specific Procedure Laboratory of the University of Louisville. In this study, normal tissues that had neuroendocrine cells (stomach, 6; small bowel, 5; colon, 6; appendix, 6; thyroid, 4; pancreas, 5; pituitary gland, 4; parathyroid, 3; parathyroid hyperplasia, 3; and adrenal, 3) and tumors derived from neuroendocrine cells, including carcinoid (stomach, 2; small bowel, 3; colon, 5; appendix, 7; fallopian tube, 1; ovary, 1; and lung, 2); atypical carcinoid (lung, 3); metastatic carcinoid (primary gut, 7); pituitary adenoma, 4; medullary carcinoma of thyroid, 4; pheochromocytoma, 2; parathyroid adenoma, 2; Merkel cell carcinoma, 6; small cell lung carcinoma, 6; large cell neuroendocrine carcinoma of lung, 4; small cell carcinoma of cervix, 2; neuroblastoma, 4; and malignant melanoma, 4, were stained with the 10H2 monoclonal antibody to CXCR2.[25] Selected sections were also stained with 2A4, a monoclonal antibody to CXCR1. These reagents, which were kindly provided by Dr Jin Kim, Genentech, Inc, South San Francisco, Calif, have previously been shown to be highly specific for their respective receptors by immunocytochemistry and flow cytometry.[24,26]

All tissues were fixed in formalin and embedded in paraffin. The sections were cut at 3 μm, treated in a microwave on high power 2 times (1 minute each for adherence to the slide), and deparaffinized through graded alcohols into water. After deparaffinization, the sections were placed into 0.5 mol/L citrate buffer at pH 5.6 and steamed for 20 minutes for epitope enhancement, except a slide for prolactin. The tissues were stained for the appropriate antigen by an automated immunohistochemical system (Ventana-Biotech, Tucson, Ariz) using their standard avidin-biotin complex method. The sections were incubated in DAB (3,3′diaminobenzidine tetrahydrochloride) as a chromogen. Double staining was performed on parathyroid and pituitary glands to determine which cell(s) express CXCR2. AEC (3-amino-9-ethylcarbazole) was used as a chromogen for CXCR2, and DAB was used for hormones (pituitary gland prolactin, growth hormone, 1:2000, polyclonal, Dako Corporation, Carpinteria, Calif; adrenocorticotropic hormone, 1:3000, polyclonal, Dako; follicle-stimulating hormone, 1:8, polyclonal, Dako; luteinizing hormone, 1:8, polyclonal, Dako; parathyroid insulin, 1:200, clone Z006, Zymed, South San Francisco, Calif; glucagon, 1:1000, polyclonal, Dako; somatostatin, 1:3000, polyclonal, Dako; and pancreatic polypeptid, 1:5000, polyclonal, Dako). Appropriate negative controls were utilized. The sections were counterstained with half-strength aqueous hematoxylin (Biomeda, Foster City, Calif) for 1 minute followed by drying and coverslipping in Permount. Cases utilizing AEC as a chromogen were coverslipped with glycergel (Dako). All cases were photographed with Nikon Microphot-FXA light microscope.

RESULTS

A panel of normal human tissues was analyzed for the expression of interleukin 8 receptors (CXCR1 and CXCR2) by immunohistochemistry. Both receptors were expressed by circulating neutrophils, which were seen in vascular spaces and in tissues. Although the expression of CXCR1 was limited to granulocytes, CXCR2 expression was noted in mononuclear phagocytic cells and in some nonhematopoietic cells.

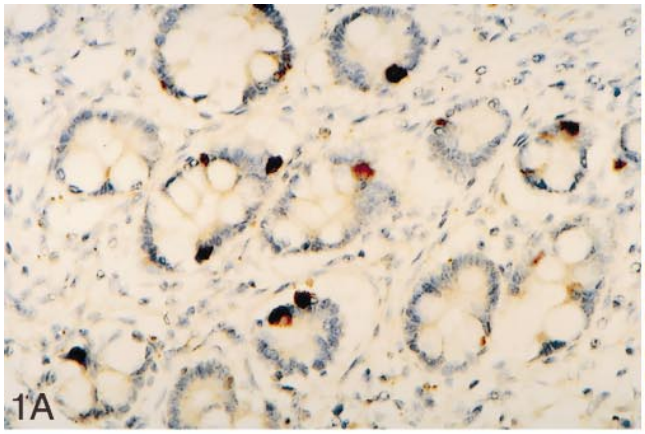
All squamous epithelium, including that of skin, esophagus, ectocervix, and vagina, showed significant expression of CXCR2 in a membranous staining pattern. Positive cells were localized to stratum spinosum of the skin and corresponding levels of the other epithelia, which were characterized by differentiating squamous cells. Cells in the basal layer and those present in the superficial, terminally differentiated region of the epithelium lacked CXCR2 activity. Epithelial cells in the stomach, small intestine, colon, liver, exocrine pancreas, lung, kidney, and adrenal cortex (zona glomerulosa and fasciculata) showed no evidence of CXCR2 expression. However, rare cells within the gastric and bronchial epithelium showed strong reactivity with the monoclonal antibody to CXCR2. Parallel analysis of adjacent sections confirmed that these cells also expressed chromogranin, which is characteristic of neuroendocrine cells.

Therefore, additional tissues were analyzed to further explore the expression of CXCR2 by neuroendocrine cells in different tissues and by their neoplastic counterparts. The results of this analysis are summarized in the Table.

Among endocrine organs, all neuroendocrine cells except parathyroid showed strong cytoplasmic reactivity for CXCR2. Kulchitsky cells in the bronchial epithelium and corresponding neuroendocrine population in the stomach, small intestine, colon, and appendix showed strong cytoplasmic staining (Figure 1, A). The adrenal medulla exhibited strong, diffuse reactivity. Besides the medulla, the zona reticularis showed a granular cytoplasmic staining pattern (Figure 1, B). All pancreatic islet cells were positive for this marker, but the intensity of immunohistochemical staining of this population was variable. Dual staining of pancreatic islets was performed to determine whether CXCR2 was preferentially expressed by one of the subpopulations of neuroendocrine cells in this structure. Alpha, beta, and delta cells, which synthesize and secrete glucagon (Figure 2, A), insulin, and somatostatin, respectively, were also positive for CXCR2, but the staining intensity varied from cell to cell. Neuroendocrine cells, which produce pancreatic polypeptide, were rare in the pancreatic islet, and it was difficult to evaluate fully their expression of CXCR2. The pituitary gland was also positive for CXCR2 expression, but, again, the staining intensity of individual cells varied. Dual staining with polyclonal antibodies to pituitary hormone and CXCR2 showed that cells from all subpopulations in the anterior pituitary gland exhibited varying degrees of CXCR2 expression (Figure 2, B). In the thyroid gland, scattered individual cells and a few groups of cells in follicles and interstitium expressed diffuse or granular cytoplasmic positivity for this marker. Cells of normal parathyroid and parathyroid hyperplasia lacked expression of CXCR2.

CXCR2 reactivity was also examined in neuroendocrine tumors arising in different tissues. They were of various levels of differentiation, such as typical carcinoid, atypical carcinoid, and small cell carcinoma, and different biologic behaviors, such as primary and metastatic carcinoids. The intensity of staining was stronger in differentiated and primary tumors than the poorly differentiated and metastatic ones.

Carcinoids from different organs (stomach, n = 2; small bowel, n = 3; colon, n = 5; appendix, n = 7 [Figure 3, A]; fallopian tube, n = 1; ovary, n = 1; and lung, n = 2) showed diffuse, strong cytoplasmic staining. Three cases of so-called atypical carcinoid, distinguished from characteristic cases by increased mitotic activity, foci of necro-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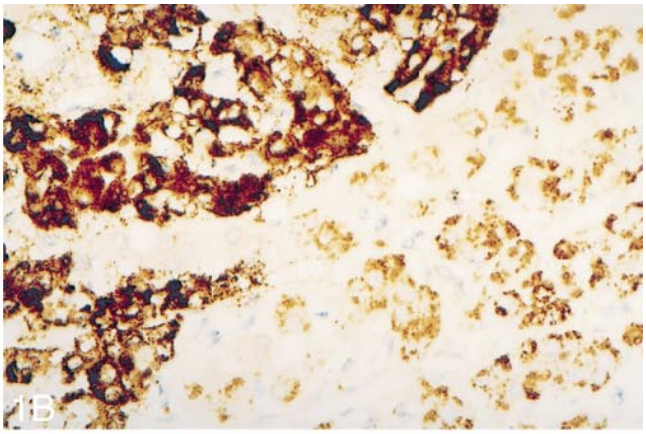
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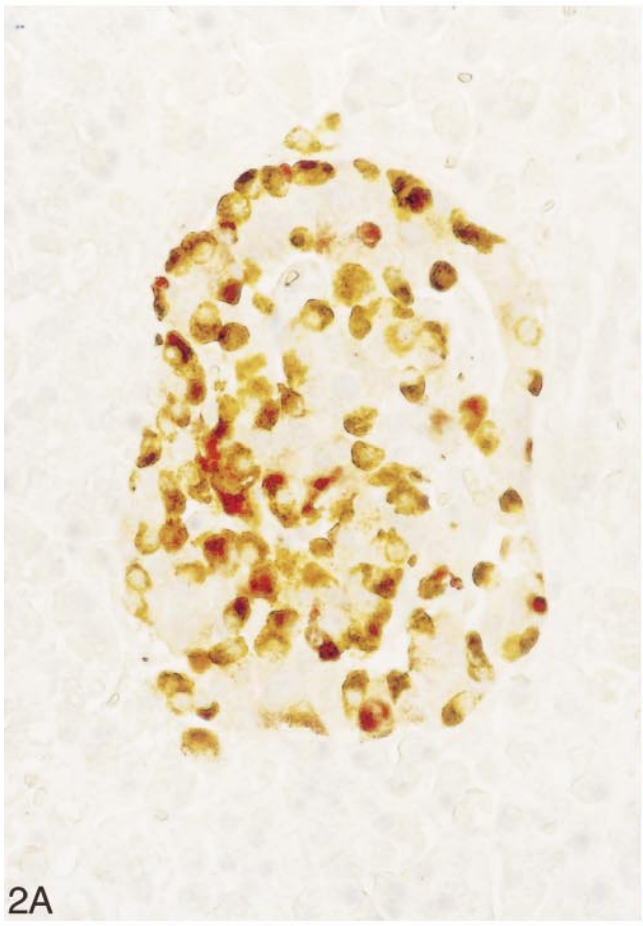
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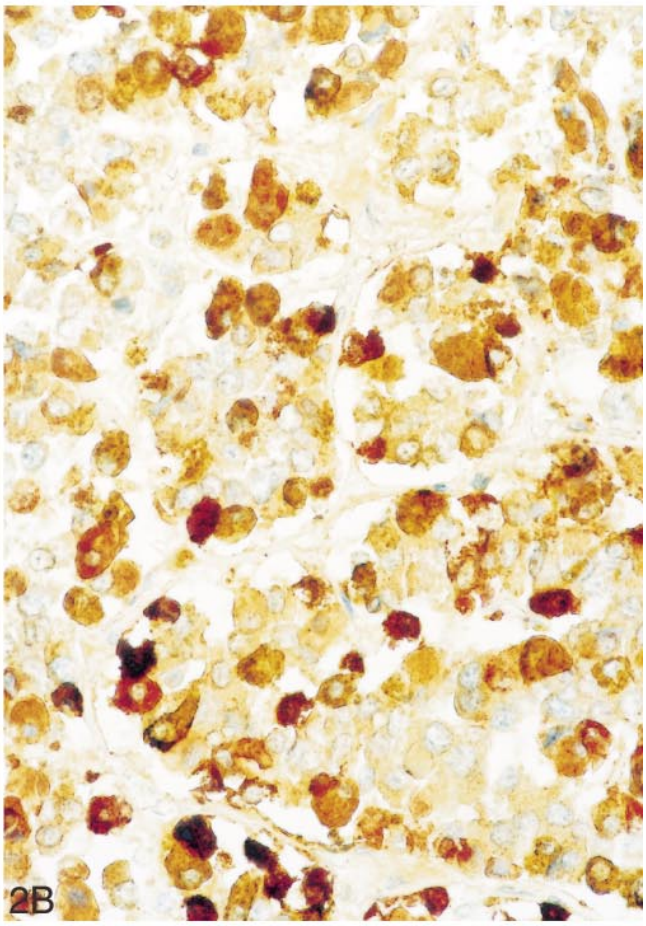
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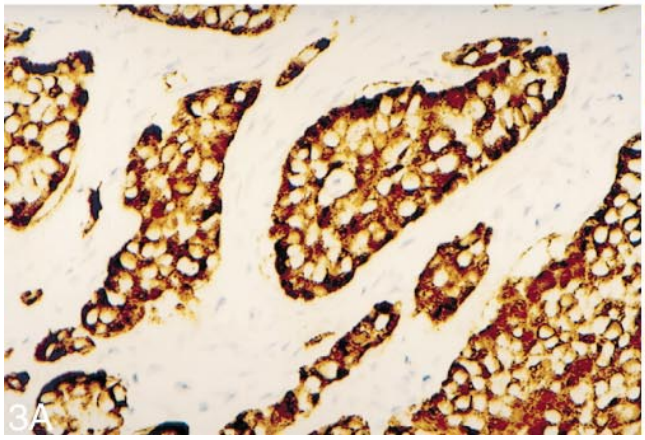
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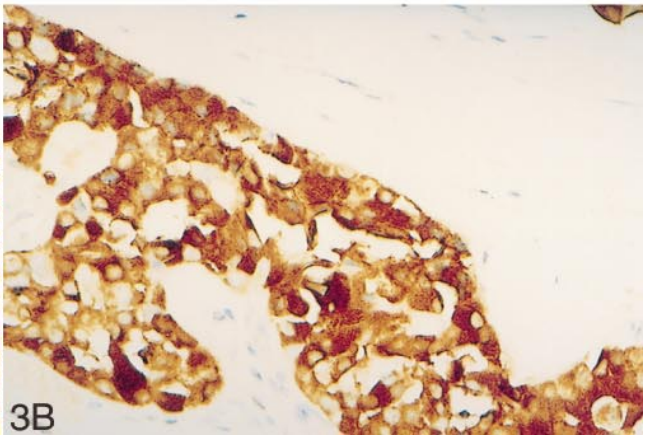
3B

CXCR2 Expression on Normal Neuroendocrine Cells and Neuroendocrine Tumors*

Cell or Tumor Type	Total No. of Cases	Intensity of Expression
Normal		
Stomach	6	3+
Small bowel	5	3+
Colon	6	3+
Appendix	6	3+
Pituitary gland	4	3+
Adrenal medulla	3	3+
Thyroid C cell	4	3+
Parathyroid	3	−
Parathyroid hyperplasia	3	−
Pancreatic islet cell	5	3+
Neoplastic		
Carcinoid		
Stomach	2	3+
Small bowel	3	3+
Colon	5	3+
Appendix	7	3+
Fallopian tube	1	3+
Ovary	1	3+
Lung	2	3+
Atypical carcinoid (lung)	3	1 to 2+
Metastatic carcinoid	7	1 to 2+
Pituitary adenoma	4	2 to 3+
MCT	4	2 to 3+
Pheochromocytoma	2	2+
Parathyroid adenoma	2	−
MCC	6	−
SCLC	6	−
LCNECL	4	−
SCC of cervix	2	−
Neuroblastoma	4	−
Malignant melanoma	4	−

* MCT indicates medullary carcinoma of the thyroid; MCC, Merkel cell carcinoma; SCLS, small cell lung cancer; LCNECL, large cell neuroendocrine carcinoma of lung; SCC, small cell carcinoma; −, not detectable; 1+, faint positive; 2+, moderate positive; and 3+, strong positive.

sis, and nuclear hyperchromasia but having morphologic, immunohistochemical, and ultrastructural features similar to carcinoid, exhibited moderate intensity of CXCR2 staining. All 7 metastatic carcinoids were also shown to have a similar level of staining. Pheochromocytoma (n = 2) showed moderate diffuse cytoplasmic staining. Pituitary adenomas (n = 4) exhibited intense diffuse cytoplasmic reactivity. Medullary carcinoma of the thyroid (n = 4) was also decorated by CXCR2, but the staining intensity varied from cell to cell (Figure 3, B). Parathyroid adenomas (n = 2), small cell lung carcinoma (n = 6), large cell neuroendocrine carcinoma of lung (n = 4), Merkel cell carcinoma (n = 6), small cell carcinoma of cervix (n = 2), neuroblastoma (n = 4), and malignant melanoma (n = 4) lacked expression of CXCR2.

COMMENT

CXCR2 originally was described in leukocytes[25,27] and then on nonhematopoietic cells, such as keratinocytes and neurons.[24,26] In this study, we show that normal neuroendocrine cells and their well-differentiated neoplastic counterparts demonstrated high-level expression of CXCR2. Normal neuroendocrine cells from the pituitary gland, zona reticularis, and medullary region of the adrenal, thyroid, pancreatic islets, gastrointestinal tract, and bronchopulmonary system showed diffuse, strong cytoplasmic staining for CXCR2. A single exception was the parathyroid gland.

Analysis of tumors derived from these neuroendocrine tissues revealed that they also expressed CXCR2. Staining intensity decreased from well-differentiated neoplasms to less differentiated ones. Although typical carcinoid expressed strong CXCR2 positivity, staining intensity was moderate in atypical carcinoid and expression was lacking in less differentiated tumors, which included small cell lung carcinoma and large cell neuroendocrine carcinoma of the lung. Gould et al emphasize the spectrum of neuroendocrine tumors of the lung in order of biologic behavior from typical carcinoid to atypical carcinoid to small cell carcinoma.[28] With this information, our data showed that CXCR2 expression correlated positively with tumor biologic behavior and differentiation. Metastatic carcinoids also expressed moderate intensity of CXCR2 positivity. Variation of staining intensity between carcinoid and metastatic carcinoid may also be related to the biologic behavior of the tumor, but further studies are necessary to clarify these issues.

Neuroendocrine cells may have different embryologic origins, including neural tube, neural crest, and endoderm. Cells from the neural crest give rise to the adrenal medulla, paraganglia, enteric ganglia, sympathetic ganglia, melanocytes, and pharyngeal arch from where thyroid C cells are derived.[29] Neuroendocrine cells of the gastroenteropancreatic and bronchopulmonary tract have been demonstrated to be of endodermal origin. Parathyroid glands also originate from endoderm of the third and fourth branchial pouches. The anterior lobe of the pituitary gland originates in a stomodeal ectoderm, and the posterior lobe arises from the neuroectodermal bud.[30] The neural tube gives rise to neuroblasts, ependymal cells, neurons, glioblasts, and glial cells.[29] Although neuroendocrine cells have different origins and morphologic features, they share a similar phenotype. They synthesize, package, and secrete biologically active neuropeptides and amines that act as paracrine regulators and modulators, neurotransmitters, and hormones.

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Figure 1. *CXCR2 expression in normal neuroendocrine cells. A, Scattered positive cells in colonic epithelium corresponding to DNS cells. B, Moderate-to-strong granular cytoplasmic staining in zona reticularis (lower right) and strong diffuse cytoplasmic staining in medullary region of the adrenal (upper left). (Immunoperoxidase, hematoxylin counterstain, ×200.)*

Figure 2. *Dual immunohistochemical staining in normal pancreatic islet and anterior pituitary gland. A, Simultaneous staining with anti-rabbit glucagon antiserum (brown) and CXCR2 (red) revealed variable staining intensity among the cells and expression of both markers in the same islet cells. B, Staining with anti-rabbit follicle-stimulating hormone antiserum (brown) and CXCR2 (red) shows positivity for both markers in some cells in pituitary gland. (Immunoperoxidase, hematoxylin counterstain, ×200.)*

Figure 3. *CXCR2 immunoreactivity in neuroendocrine neoplasms. A, Strong cytoplasmic positivity in a carcinoid arising in the appendix. B, Faint-to-strong cytoplasmic positivity in medullary carcinoma of the thyroid. (Immunoperoxidase, hematoxylin counterstain, ×200.)*

Although neuroendocrine cells may be distinguished based on their secretory products, this approach is limited by the multiplicity of neuropeptides and the variability of their expression. Neuron-specific enolase, a cytoplasmic enzyme, was the first immunohistologic marker to identify neuroendocrine cells. It is expressed by a broad spectrum of neuroendocrine tumors.[5–8] Its expression is particularly important in poorly differentiated neuroendocrine neoplasms, because, in general, other neuroendocrine markers are less likely to be positive in this group of tumors.[7,8,31–34]

Neuron-specific enolase is expressed not only by neuroendocrine and neuroectodermal cells and tumors but also by other cells and neoplasms,[35,36] thus limiting its practical utility as a neuroendocrine marker. However, it has been reported that use of monoclonal neuron-specific enolase antibodies decreases this limitation and provides higher specificity for neuroendocrine tumors.[37] Synaptophysin is another neuroendocrine marker that is used widely and is expressed in all neural-type neuroendocrine tumors, whether well differentiated or poorly differentiated.[11] Synaptophysin is expressed in epithelial types of neuroendocrine cells and their neoplastic counterparts, such as all variants of islet cell tumors, medullary thyroid carcinomas, several adenomas of the pituitary gland and the parathyroid, and most carcinoids from different primary sites.[10–12] It is less frequently positive in poorly differentiated neuroendocrine carcinomas, such as small cell lung carcinoma and Merkel cell carcinoma, than in those that are well differentiated.[10,11,38] Another neuroendocrine marker, CD56, is expressed in normal and neoplastic neuroendocrine tissues except for the parathyroid. This tissue was also the only exception in our study. CD56 is positive in well-differentiated and poorly differentiated neuroendocrine tumors.[13,39]

Chromogranin A is also a specific and reliable marker. This marker has been shown in normal pituitary glands and adenomas, the adrenal medulla and pheochromocytomas, pancreatic islet cells predominantly in alpha cells, a normal parathyroid and adenomas, Merkel cell carcinoma, and carcinoids of gastrointestinal and pulmonary tissues.[9,10] It is expressed in well-differentiated neuroendocrine tumors more than in poorly differentiated ones, such as small cell lung carcinoma and neuroblastoma.[7,9,10]

Chromogranin was also demonstrated in the lymphoreticular system by immunohistochemistry. These results are one part of an increasing body of evidence that suggests that there is a connection between the immune system and the neuroendocrine system.[40] Although their precise role in neuroendocrine function has not been elucidated, chemokines have previously been shown to influence this system.[41] Rat CINC/gro, which is a C-X-C chemokine that binds CXCR2, induced prolactin, growth hormone, and adrenocorticotropic hormone but suppressed luteinizing hormone and follicle-stimulating hormone secretion from normal rat anterior pituitary gland cells in vitro.[41] CINC/gro was also demonstrated to be expressed in the anterior pituitary gland of the rat by immunohistochemistry.[42] It has been reported that melanoma cases (7 of 10) expressed MGSA/GRO and its receptor CXCR2.[19] This chemokine may have autocrine effects on melanoma cells and promote cell growth and paracrine effects that are angiogenic on endothelial cells, resulting in enhancement of tumor growth and, perhaps, metastasis.[19] Interleukin 8 has also been found to be expressed in melanoma and gastric carcinoma and may regulate tumor growth as well.[20,21] It is now clear that chemokines have functions besides the induction of the directed migration of leukocytes. They may play a role in the modulation of angiogenesis and tumor growth. The findings of the present study may represent a link between the immune and neuroendocrine systems and raise the possibility of a novel function for chemokines in neuroendocrine cell biology.

This is the first demonstration of the expression of CXCR2 in neuroendocrine cells and their neoplastic counterparts. The positive staining in neuroendocrine cells and benign and low-grade neuroendocrine tumors and the absence of negative staining in high-grade neuroendocrine malignant neoplasms suggest that CXCR2 expression may be related to the function of terminally differentiated cells but not tumor development. The absence of neuroendocrine abnormalities in mice nullizygous for CXCR2 through gene targeting indicates that the chemokine ligands of this receptor are not involved in the embryologic development of neuroendocrine tissues.[43]

This study may add to the armamentarium of diagnostic reagents for normal neuroendocrine cells and tumors. Further studies may provide insight into the precise role of this receptor in neuroendocrine cells.

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