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Nerve growth factor release with mast-cell-derived mediators in a patient with systemic mastocytosis after middle-wave ultraviolet irradiation

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Key words: cytokine; mediator; nerve growth factor; systemic mastocytosis; ultraviolet light.

Background: The symptoms of a 56-year-old man with systemic mastocytosis became worse with exposure to sunlight. We evaluated mast-cell-derived mediators and cytokines before and after exposure to ultraviolet light in the patient.

Methods: The patient was irradiated with middle-wave ultraviolet light, so-called ultraviolet light B, and the levels of mediators and cytokines were measured serially. The point mutation Asp816Val in *c-kit* was investigated by analyzing polymerase chain reaction products from the complementary DNA of peripheral blood mononuclear cells.

Results: Before irradiation, the levels of mast-cell-derived mediators and metabolites were elevated. Among various cytokines measured, including soluble *c-kit* and stem cell factor, only the level of nerve growth factor was elevated. After irradiation, the nerve growth factor level was further increased along with the levels of mast-cell-derived mediators and metabolites. The point mutation Asp816Val in *c-kit* was not detected in peripheral blood mononuclear cells.

Conclusions: Middle-wave ultraviolet light may activate mast cells to release nerve growth factor and mediators in systemic mastocytosis.

Date:

Accepted for publication 18 May 1999

To cite this article:

Kurosawa M., Inamura H., Amano H., Kanbe N., Nagata H., Nagai H., Furukawa S. & Miyachi Y. Nerve growth factor release with mast-cell-derived mediators in a patient with systemic mastocytosis after middle-wave ultraviolet irradiation. *Allergy* 1999; **54**, 994–998.

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ISSN 0105-4538

Mastocytosis consists of an array of clinical and biochemical abnormalities that arise from tissue infiltration by an excess of mast cells and the release of various biologically active mediators from the cells (1). However, we have very little insight into the nature of the physical factors that activate mast cells in this disease.

The biologic effects of ultraviolet light are of particular interest, because it has been shown that irradiation at $>200 \text{ mJ/cm}^2$ with middle-wave ultraviolet light, so-called

ultraviolet light B (UVB), induces mast-cell degranulation in the skin (2), and human skin explants respond to UVB irradiation by increasing the synthesis of prostaglandins (3). UVB irradiation has also recently been demonstrated to induce human skin mast-cell degranulation *in vivo* and *in vitro* (4).

In this paper, we report a patient with systemic mastocytosis whose history suggested that exposure to sunlight could worsen his symptoms. To evaluate this possibility, he was exposed to UVB and the changes of various mediators and cytokines were investigated serially. This study demonstrated that the plasma level of nerve growth factor (NGF) was increased in systemic mastocytosis, and that exposure to UVB induced further elevation of NGF in parallel with mast-cell-derived mediators, suggesting that UVB may activate mast cells in systemic mastocytosis.

Case report

A 56-year-old man was admitted to the Department of Dermatology at Gunma University Hospital (Japan) in November 1997 with widespread pruritic skin lesions on the trunk and extremities consisting of brown-red macules. He also complained of serous rhinorrhea and dyspepsia. About 10 years previously, he had noted the first episode of flushing and edema of the legs after prolonged exposure to sunlight. Since then, the skin lesions had appeared and slowly spread over the years. He also stated that prolonged exposure to sunlight made his symptoms worse. Physical examination revealed urticaria pigmentosa and Darier's sign.

Biopsy specimens were obtained and immediately fixed in Carnoy's fluid at room temperature. Bone-marrow biopsy specimens from the posterior iliac crest were fixed in Carnoy's fluid and decalcified for embedding. Tissues were embedded in paraffin, and 4- μ m-thick sections were prepared for immunohistochemical studies to detect human mast cells using monoclonal murine antitryptase and antichymase antibodies (kindly provided by Prof. L.B. Schwartz, Virginia Commonwealth University, Richmond, VA, USA), which were originally reported by Irani et al. (5). The bone-marrow biopsy specimens showed inhomogeneous focal infiltration of mast cells in the peritrabecular and perivascular areas. Biopsy specimens from skin lesions, nasal mucosa, bronchus, and a gastric lesion showed an increase of mast cells in each specimen. No findings

indicating allergy were observed, and a diagnosis of systemic mastocytosis was made.

Material and methods

UVB irradiation

Written informed consent from the patient was obtained after the nature and possible consequences of the study had been fully explained. The study was approved by the Institutional Ethics Committee of Gunma University School of Medicine. Before and after the UVB irradiation test, the patient was not receiving any medications.

The experimental light was middle-wave ultraviolet light, conventionally designated as UVB. Dose-response thresholds for UVB-induced erythema on non-sun-exposed buttock skin were obtained to establish the minimal erythemogenic dose (MED) (6). The source of UVB was 120-W sunlamp tubes (FL20S.E, Toshiba Light and Technology, Tokyo, Japan) emitting wavelengths of 275–410 nm, with peak emission at 315 nm (mainly UVB). The radiant intensity was measured by a Digital Radiometer J-260 (Ultraviolet Products, Inc., San Gabriel, CA, USA), and was 0.85 mW/cm² in the UVB range at a distance of 20 cm. We have recently demonstrated that 200 mJ/cm² is the maximum dose of UVB which did not cause significant histamine release from mast cells (7); therefore, if we take this finding together with others (2, 4), the patient's back was exposed to UVB at a dose of 200 mJ/cm² (3 MED) over an area of approximately 0.2 m².

Assay of mediators

Plasma histamine and urine *N*-methylhistamine were determined by radioimmunoassay (RIA) (8) (Immunotech, Marseille, France) and double antibody RIA (9) (Pharmacia & Upjohn, Uppsala, Sweden), respectively. Serum tryptase was determined with a tryptase RIA kit (Pharmacia & Upjohn) (10). Urinary creatinine (Cr) was determined with an L-type Creatinine-F assay system (Wako Pure Chemical, Osaka, Japan), and urinary levels of mediators were expressed per milligram of Cr to correct for the volume of urine voided.

For the measurement of leukotriene C₄ (LTC₄), heparinized whole blood was mixed with cold ethanol, and the supernatant was obtained after centrifugation. The sample was mixed with sodium phosphate buffer and dichloromethane. After centrifugation, the upper water layer was loaded onto a Sep-Pak C18 cartridge (Waters, Milford, MA,

USA). The LTC₄ fraction was eluted and dried, and the assay was performed with a [³H]LTC₄ assay system (Amersham, Buckinghamshire, UK).

For the measurement of LTE₄, urine was mixed with a buffer containing ethylenediaminetetraacetic acid (EDTA) and was centrifuged. The supernatant was spiked with 3000 dpm of [³H]LTE₄ as an internal standard and was loaded onto a Sep-Pak C18 cartridge. Leukotrienes were eluted, dried, and reconstituted with the high-performance liquid chromatography mobile phase. Separation was performed on a TSK-ODS-120T column (Tosoh, Tokyo, Japan). Fractions were collected, evaporated to dryness, and reconstituted with assay buffer (0.1 M phosphate buffer, pH 7.5, containing 0.9% sodium chloride, 0.1% bovine serum albumin, and 0.5% preservative). A sample was used to check ³H radioactivity for the estimation of recovery. The assay was performed with a [³H]LTC₄/D₄/E₄ assay system (Amersham).

To measure prostaglandin D₂ (PGD₂), whole blood was collected into a glass syringe containing EDTA and indomethacin. After centrifugation, the plasma was mixed with acetic acid and applied to a C2 column (Amersham). The fractions containing PGD₂ were eluted and dried, and the assay was performed with a [³H]PGD₂ assay system (Amersham).

All assays of each sample were performed in triplicate, and the results are reported as mean values.

Assay of plasma cytokines

Interleukin-3 (IL-3), IL-4, IL-5, IL-13, macrophage inflammatory protein-1 α (MIP-1 α), tumor necrosis factor- α (TNF- α), soluble *c-kit* (cKIT), and stem cell factor (SCF) were measured with the following enzyme-linked immunosorbent assay kits: IL-3 (R&D Systems, Minneapolis, MI, USA), IL-4 (BioSource, Camarillo, CA, USA), IL-5 (Amersham), IL-13 (Bender MedSystem, Vienna, Austria), MIP-1 α (Amersham), TNF- α (R&D Systems), cKIT (Nichirei, Tokyo, Japan), and SCF (Amersham). NGF level was measured by a highly sensitive, two-site, enzyme immunoassay system for human and murine NGF that could detect concentrations as low as 1 pg/ml (11).

All assays of each sample were performed in triplicate, and the results are reported as mean values.

Identification of a point mutation in *c-kit*

The point mutation Asp816Val in *c-kit* was investigated by analyzing polymerase chain reaction products from the

complementary DNA (cDNA) of peripheral blood mononuclear cells (12).

Results

Before UVB irradiation, measurement of mediators in blood and urine showed elevated levels of plasma histamine, urinary methylhistamine, serum tryptase, plasma LTC₄, and urinary LTE₄. Plasma PGD₂ level was normal. Among plasma cytokines, the NGF level was elevated, but the other cytokines were normal. After UVB irradiation, plasma histamine level was increased at 15 min and 48 h in parallel with an increase of urinary methylhistamine. Serum tryptase level was increased at 15 min and 5 h. Plasma LTC₄ level was also increased at 15 min and 5 h, remaining elevated until 48 h. Urinary LTE₄ was increased at 15 min, 24 h, and 48 h. Plasma PGD₂ level was increased at 15 min. Among the cytokines measured, only NGF was increased at 15 min and 5 h with an elevated level persisting until 48 h (Table 1). About 10–30 min and 5 h after irradiation, the patient complained of itching of the irradiated site, but no systemic reaction occurred.

Investigation of the *c-kit* Asp816Val activating mutation in the patient's peripheral blood mononuclear cells by analyzing polymerase chain reaction products of cDNA revealed no evidence of this mutation (Fig. 1).

Discussion

Systemic mastocytosis is characterized by abnormal proliferation of tissue mast cells, and its symptoms are primarily attributed to the release of mediators by these cells during episodes of activation. Although it is important to exclude an allergic origin for mast-cell activation, an allergic basis is identified only in a few patients.

Our patient had constitutional symptoms, urticaria pigmentosa, and an increase of mast cells in biopsy specimens along with elevated levels of mast-cell-derived mediators in blood and urine; therefore, he fulfilled the criteria for systemic mastocytosis (1). Among the various cytokines measured, only the NGF level was elevated. The history of our patient suggested that exposure to sunlight could worsen his symptoms. To investigate whether exposure to sunlight activated cells in the skin, including mast cells, he was subjected to UVB irradiation, and the levels of mediators and cytokines in the blood and urine were measured serially. After UVB irradiation, blood and

Table 1. Changes of levels of mediators in blood and urine, and plasma cytokines in patient with systemic mastocytosis after irradiation with middle-wave ultraviolet light

	Time					Normal range
	Before	15 min	5 h	24 h	48 h	
Histamine (ng/ml)	4.0	43.5	12.7	8.7	31.2	0.05–0.18 (n=98)
Urine methylhistamine (ng/mg Cr)	9410	13 900	9490	19 100	12 300	65–280 (n=20)
Tryptase (ng/ml)	3.3	5.5	5.5	4.3	3.8	<2.0 (n=20)
LTC ₄ (pg/ml)	25.9	68.1	60.8	46.3	38.1	<13.0 (n=12)
Urine LTE ₄ (pg/mg Cr)	2310	3830	2370	3500	3860	50–450 (n=10)
PGD ₂ (pg/ml)	31.5	60.1	23.5	31.3	32.9	25.0–43.0 (n=10)
IL-3 (pg/ml)	0.52	<0.5	<0.5	<0.5	<0.5	<0.65 (n=20)
IL-4 (pg/ml)	<0.5	<0.5	<0.5	<0.5	<0.5	<3.61 (n=20)
IL-5 (pg/ml)	<7.8	<7.8	<7.8	<7.8	<7.8	<7.8 (n=20)
IL-13 (pg/ml)	6.86	7.69	8.33	8.45	7.21	<28.6 (n=20)
MIP-1 α (pg/ml)	<46.9	<46.9	<46.9	<46.9	<46.9	<46.9 (n=47)
TNF- α (pg/ml)	4.06	4.22	3.99	4.73	3.88	1.52–12.0 (n=30)
cKIT (A.U./ml)	263	291	276	283	290	181–297 (n=97)
SCF (pg/ml)	872	861	1180	920	912	920–1402 (n=97)
NGF (pg/ml)	6.75	52.8	38.1	11.2	7.45	<5.5 (n=10)

After irradiation with middle-wave ultraviolet light at dose of 200 mJ/cm², levels of mediators in blood and urine increased in different manners. Among plasma cytokines measured, only level of nerve growth factor was increased after irradiation.

urine levels of mast cell-derived mediators were increased. Among the plasma cytokines measured, however, only NGF was increased.

We cannot conclude whether mast-cell activation alone was responsible for the increase of mediators in our patient because other skin cells could also be affected by UVB irradiation. However, the increased levels of mast-cell-derived mediators, such as histamine, methylhistamine, tryptase, PGD₂, LTC₄, and LTE₄, strongly suggested that dermal mast cells were activated by UVB irradiation,

resulting in degranulation to release stored mediators and the generation and release of lipid mediators.

Recently, there have been some advances in our understanding of mast-cell development and maturation. SCF is the ligand of the proto-oncogene *c-kit* and it has been recognized to have an important effect on the growth of mast cells (13). Increased expression of the soluble form of SCF has been reported in the skin of patients with systemic mastocytosis, suggesting that overproduction of SCF might play a role in this disorder (14). In our patient, however, the levels of cKIT and SCF were normal before and after irradiation. On the other hand, a point mutation of *c-kit* has been recognized in mastocytosis patients, a feature which helps to explain the mast-cell hyperplasia in the disease (6, 15), but the *c-kit* Asp816Val activating mutation was not noted in our patient. However, this does not exclude other mutation in *c-kit*. Our results suggest that overproduction of SCF may not always be present in systemic mastocytosis and that the Asp816Val mutation may be a permissive mutation for the disease.

Interestingly, among the cytokines measured, only NGF was elevated before UVB irradiation, and its level was increased after irradiation. NGF is a well-characterized neurotrophic factor that is essential for the survival, development, and maintenance of peripheral sympathetic

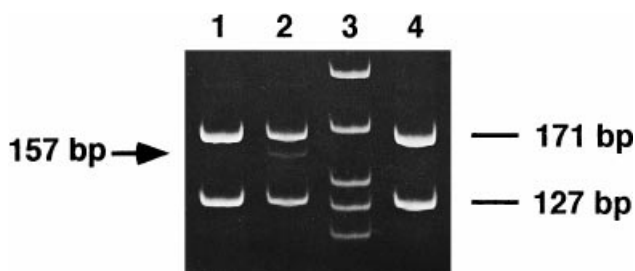


Figure 1. Detection of activating *c-kit* mutation at nucleotide 2468 by cleaving polymerase chain reaction products with *Hinf* I and *Hae* III. Lane 1) normal control; lane 2) positive control, cDNA from skin lesion of patient with systemic mastocytosis who has mutation; lane 3) size markers (242, 190, 147, 124, and 110 bp from top to bottom); lane 4) our patient. Only lane 2 shows aberrant 157-bp band, indicating that peripheral blood mononuclear cells from our patient are negative for mutation.

and embryonic sensory neurons (16). Injection of NGF into neonatal rats increases the number of mast cells in various tissues (17), and NGF induces the development of connective-tissue-type mast cells from murine bone-marrow cells *in vitro* (18). It has also been shown that NGF promotes the survival of rat peritoneal mast cells (19), and that rat peritoneal mast cells synthesize, store, and release NGF (20). Taken together, these observations suggest that NGF may play a role in connective-tissue-type mast cells. It remains unclear whether mast-cell activation alone was responsible for the increased level of NGF in our patient, because cultured human keratinocytes have been shown to express NGF mRNA and secrete NGF (21). However, elevation of the NGF level occurred in parallel with the increase of mast-cell-derived mediators, and our most recent studies have indicated that cultured human mast cells express NGF mRNA (unpublished data). Accordingly, the present data

strongly suggest that human mast cells secrete NGF, and an autocrine influence of NGF on human mast-cell function, growth, and differentiation should be considered. To our knowledge, this is the first report of increased plasma NGF in a patient with systemic mastocytosis. Our findings need to be confirmed in more patients.

In conclusion, this study demonstrated for the first time that UVB irradiation induced elevation of the levels of mast-cell-derived mediators and NGF in a patient with systemic mastocytosis, an effect which may suggest a pathophysiologic role of these agents in the disease.

Acknowledgments We are grateful for technical assistance by Mr Tetsuji Yamashita and Mr Fumihiko Kurimoto at Research and Development, Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo, Japan.

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