

immunonegative for CM2B4 and contained no MCPyV, based on PCR of the bulk tissue comprising MCC and SCC. Based on these immunohistochemical and ultrastructural characteristics, we diagnosed the patient with a collision tumor comprising MCC and SCC.

The present case is a collision tumor comprising MCC and SCC, showing no histopathologic signs of a transition between tumors. MCC is an uncommon tumor that is not yet well understood. A significant proportion of MCC occurs in close association with malignant epithelial neoplasms, mainly SCC as in the present case [3]. Because MCC and SCC are both ultraviolet-induced neoplasms, the occurrence of the two cutaneous malignancies is not unexpected. MCC exhibits divergent histopathologies, such as squamous, eccrine, and melanocytic differentiation, both in primary and metastatic sites. The histogenesis of MCC remains controversial, but some reports suggest that the concurrent development of MCC and squamous neoplasms in the same lesion supports the theory of a pluripotent epidermal stem cell origin. MCC shows unique histopathologic features that have prompted reconsideration of the histogenesis of the lesion. Although interleukin-1, interleukin-6, tumor necrosis factor, and vascular endothelial growth factor are involved in tumor immunology and tumorigenesis, the mechanism underlying the synchronous appearance of the malignant tumors is unknown.

In addition, the present case demonstrated an immunohistochemical profile of dermal neoplastic cells that was distinctively positive for CD56 and TTF-1, and negative for CK20, LCA, and S-100. MCC displays positive immunohistochemical reactions to CK20 and neural cell adhesion molecule (NCAM/CD56) [4]. TTF-1 is also expressed, however, in SCCL, pulmonary carcinoids, and non-SCCL [5]. TTF-1 is a sensitive and specific marker for SCCL. We have detected the immunohistochemical expression of TTF-1 in MCC, SCC, and SCLC (Table 1) [6,7].

Until recently, MCC had been consistently shown to stain negative for TTF-1. Therefore, this stain is commonly used in conjunction with CK20, one of the most important immunophenotypic markers of MCC, to distinguish MCC from cutaneous metastasis of SCCL [8]. Our literature search, however, revealed two exceptional cases of TTF-1-positive MCC with CK20 positivity [9,10]. Bobos et al. [6] reported that CK20 was expressed in the majority of MCC (339/391; 86.7%) and CK20 was expressed in 14/302 (4.6%) of SCCL cases [6].

CD56 staining is almost as sensitive as CK20 staining for the detection of MCC (90.6% cases labeled with CD56 compared to 86.7% with CK20) [6,7]. Most MCC stain positive for CK20 and CD56, and negative for TTF-1 [5]. To our knowledge, there are no published reports of MCC staining negative for CK20 and positive for TTF-1. We report a rare variant of MCC that is CK20 negative, CD56 positive, and TTF-1 positive. It is unclear whether this collision tumor involving the specific subtype of MCC affected the expression of CK20 and TTF-1. Therefore, this MCC variation should be included in the differential diagnosis of neoplasms that stain negative for CK20 negative and positive for TTF-1. With the rising

incidence of MCC, novel findings of this disease will become more common in the future.

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Shinichi Koba*
Takuya Inoue
Takeshi Okawa
Akiko Tara

*Division of Dermatology, Department of Internal Medicine,
Faculty of Medicine, Saga University, Saga, Japan*

Naomi Yada
*Department of Pathology, Faculty of Medicine,
Oita University, Yufu, Japan*

Noriyuki Misago
Yutaka Narisawa
*Division of Dermatology, Department of Internal Medicine,
Faculty of Medicine, Saga University, Saga, Japan*

*Corresponding author at: 5-1-1 Nabeshima, Saga,
Saga 849-8501, Japan.
Tel.: +81 0952 34 2368;
fax: +81 0952 34 2017
E-mail address: kobashi@cc.saga-u.ac.jp (S. Koba).

21 November 2010

doi:10.1016/j.jdermsci.2011.06.011

Letter to the Editor

Plasma platelet activation markers in patients with atopic dermatitis and concomitant allergic diseases

Apart from a well-established role of platelets in haemostasis, their activation may contribute to the development of chronic allergic inflammation [1]. Platelets release a number of potent

mediators of vasodilatation and increased vascular permeability [2], and chemoattractants promoting migration of leukocytes to the site of allergic inflammation [3]. Atopic dermatitis (AD) is a chronic skin disorder of the complex pathogenesis involving immediate hypersensitivity and cellular responses, characterized by skin inflammation and itching [4]. We hypothesized that in AD

Table 1

Platelet count and markers in atopic dermatitis patients vs. healthy controls (A); and in atopic dermatitis patients only, subgrouped with regard to the (accompanying) phenotype (B–E) or disease severity (F).

A. Platelet count and markers in atopic dermatitis patients and healthy controls			
	Atopic dermatitis patients (n = 143)	Healthy controls (n = 71)	P-value
Platelet count ($10^3/\mu\text{L}$)	236.0 [240.0]	193.0 [286.0]	0.00001 (0.00002) ^a
PF4 (ng/mL)	22.9 [41.0]	7.5 [7.6]	<0.000001 (<0.000001) ^b
β -TG (ng/mL)	55.0 [80.8]	15.3 [16.9]	<0.000001 (<0.000001) ^b
VEGF (pg/mL)	54.9 [51.5]	39.1 [27.7]	<0.000001 (<0.000001) ^b
PDGF (pg/mL)	302.0 [434.0]	115.0 [364.0]	<0.000001 (<0.000001) ^b
B. Accompanying asthma			
	No asthma (n = 104)	Asthma (n = 39)	P-value
Platelet count ($10^3/\mu\text{L}$)	231.5 [221.0]	266.0 [230.0]	0.13
PF4 (ng/mL)	21.2 [41.0]	27.3 [17.5]	0.03 (<0.05) ^b
β -TG (ng/mL)	50.7 [80.8]	65.3 [55.8]	0.04 (0.08) ^b
VEGF (pg/mL)	53.8 [48.7]	59.8 [51.1]	0.23
PDGF (pg/mL)	281.0 [422.0]	341.0 [396.0]	0.007 (0.02) ^b
C. Coexisting allergic rhinitis			
	No allergic rhinitis (n = 67)	Allergic rhinitis (n = 76)	P-value
Platelet count ($10^3/\mu\text{L}$)	231.0 [238.0]	249.5 [205.0]	0.44
PF4 (ng/mL)	18.9 [41.0]	29.4 [32.9]	<0.000001 (<0.000001) ^b
β -TG (ng/mL)	43.2 [67.8]	67.7 [66.7]	<0.000001 (<0.000001) ^b
VEGF (pg/mL)	45.5 [48.7]	64.7 [51.1]	<0.000001 (<0.000001) ^b
PDGF (pg/mL)	236.0 [366.0]	352.5 [396.0]	<0.000001 (<0.000001) ^b
D. Coexisting allergic conjunctivitis			
	No allergic conjunctivitis (n = 92)	Allergic conjunctivitis (n = 51)	P-value
Platelet count ($10^3/\mu\text{L}$)	233.5 [240.0]	250.0 [221.0]	0.24
PF4 (ng/mL)	20.3 [41.0]	28.3 [23.3]	0.01 (0.02) ^b
β -TG (ng/mL)	51.2 [73.4]	62.7 [66.7]	0.18
VEGF (pg/mL)	49.4 [51.5]	63.7 [45.0]	0.00005 (0.00007) ^b
PDGF (pg/mL)	273.5 [434.0]	341.0 [345.0]	0.03 (0.04) ^b
E. Coexisting food allergy			
	No food allergy (n = 89)	Food allergy (n = 54)	P-value
Platelet count ($10^3/\mu\text{L}$)	235.0 [240.0]	239.0 [205.0]	0.89
PF4 (ng/mL)	20.1 [41.0]	29.2 [23.3]	0.002 (0.002) ^b
β -TG (ng/mL)	47.3 [70.0]	67.4 [59.4]	0.000004 (0.000003) ^b
VEGF (pg/mL)	48.9 [51.2]	60.8 [48.7]	0.00009 (0.00006) ^b
PDGF (pg/mL)	265.0 [434.0]	341.0 [378.0]	0.0002 (0.0001) ^b
F. Mild-to-moderate AD (SCORAD ≤ 50) vs. severe AD (SCORAD > 50)			
	SCORAD ≤ 50 (n = 125)	SCORAD > 50 (n = 18)	P-value
Platelet count ($10^3/\mu\text{L}$)	236.0 [221.0]	250.5 [224.0]	0.60
PF4 (ng/mL)	24.0 [33.9]	20.0 [37.7]	0.84
β -TG (ng/mL)	55.0 [80.8]	52.2 [52.3]	0.99
VEGF (pg/mL)	55.7 [48.7]	45.1 [51.2]	0.06
PDGF (pg/mL)	304.0 [434.0]	280.5 [322.0]	0.77

PF4, platelet factor 4; β -TG, β -thromboglobulin; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; SCORAD, severity scoring of atopic dermatitis index.

^a Adjusted for age, sex and smoking status.

^b Adjusted for age, sex, smoking status and platelet count.

Table 2

Final regression models showing significant independent effects of allergic disorders or atopic sensitization on platelet count on platelet markers in atopic dermatitis patients and in the whole study population.

	Platelet count ^a	PF4 ^b	β -TG ^b	VEGF ^b	PDGF ^b
A.					
Independent predictors: asthma, allergic rhinitis, allergic conjunctivitis, food allergy, SCORAD, atopic sensitization ^c and total serum IgE		1. Allergic rhinitis; 0.53 [0.09], β [SE]; $P < 0.000001$	1. Allergic rhinitis; 0.47 [0.09], β [SE]; $P < 0.000001$ 2. Food allergy; 0.25 [0.08], β [SE]; $P = 0.002$	1. Allergic rhinitis; 0.39 [0.09], β [SE]; $P = 0.00003$ 2. Food allergy; 0.19 [0.08], β [SE]; $P = 0.02$	1. Allergic rhinitis; 0.48 [0.09], β [SE]; $P < 0.000001$ 2. Food allergy; 0.18 [0.08], β [SE]; $P = 0.02$

Table 2 (Continued)

	Platelet count ^a	PF4 ^b	β -TG ^b	VEGF ^b	PDGF ^b
B.					
Independent predictors: AD, asthma, allergic rhinitis, allergic conjunctivitis, food allergy, atopic sensitization ^c and total serum IgE	1. AD; 0.22 [0.08], β [SE]; $P=0.01$	1. AD; 0.65 [0.04], β [SE]; $P<0.000001$ 2. Allergic rhinitis; 0.33 [0.05], β [SE]; $P<0.000001$	1. AD; 0.42 [0.05], β [SE]; $P<0.000001$ 2. Allergic rhinitis; 0.39 [0.06], β [SE]; $P<0.000001$ 3. Allergic conjunctivitis; -0.12 [0.05], β [SE]; $P=0.02$ 4. Food allergy; 0.20 [0.05], β [SE]; $P=0.00006$	1. AD; 0.32 [0.06], β [SE]; $P=0.000001$ 2. Allergic rhinitis; 0.34 [0.07], β [SE]; $P=0.000002$ 3. Food allergy; 0.14 [0.06], β [SE]; $P=0.01$	1. AD; 0.48 [0.06], β [SE]; $P<0.000001$ 2. Allergic rhinitis; 0.35 [0.06], β [SE]; $P<0.000001$ 3. Food allergy; 0.13 [0.05], β [SE]; $P=0.01$

Final regression models showing significant independent effects of allergic disorders or atopic sensitization on platelet count and platelet markers in atopic dermatitis patients (A) and in the whole study population (B) AD, atopic dermatitis; other abbreviations – see Table 1.

^a Adjusted for age, sex and smoking.

^b Adjusted for age, sex, smoking and platelet count.

^c Defined as a presence of sensitization to at least one of the four allergens tested (see main text or Supplementary Table 1).

subjects, the presence of concomitant allergic disorder might enhance platelet activation.

Therefore, platelet activation markers, including β -thromboglobulin (β -TG), platelet factor-4 (PF-4), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), were measured in a large group of 143 consecutive AD patients in remission, with or without asthma, allergic rhinitis (AR), allergic conjunctivitis (AC) and/or food allergy (FA), and compared with the results obtained in 71 healthy age- and sex-matched controls. Detailed description of the population, clinical criteria, laboratory investigations and statistical analysis can be found in [Supplementary materials](#).

Plasma PF-4, β -TG, VEGF and PDGF levels were substantially elevated in patients with AD compared to healthy controls (Table 1). Analysis of platelet markers in AD patients with concomitant allergic disorders showed that AD patients with accompanying AR demonstrated higher levels of all four platelet markers compared to non-AR subjects with AD (Table 1). A similar increase of all platelet activation markers was observed in AD patients with FA (Table 1). AD patients with concomitant asthma presented higher plasma levels of PF-4 and PDGF, while AD patients with AC had increased levels of PF-4, VEGF, and PDGF compared with the remaining subjects (Table 1). There was no association between plasma levels of platelet activation markers and AD severity (SCORAD; Table 1). Atopic sensitization was not associated with plasma levels of any platelet activation marker in either AD patients or healthy controls (not shown).

To determine the independent predictors of the platelet activation markers, two final regression models were constructed. Within the AD group ($n=143$; Table 2A), only AR and FA were independent predictors of platelet markers. Analysis of whole study group ($n=214$; Table 2B) also demonstrated AR and FA to be the independent predictors of platelet markers levels. Of importance, AD was a potent independent predictor of platelet count and the platelet activation markers. A weak negative effect on β -TG was also observed for AC and no independent effect was shown for asthma. Therefore, the results of the final models (Table 2) showed that the positive associations between AC or asthma (marginal) and platelet activation markers observed in direct comparisons (Table 1), resulted from potent independent effects of coexisting AR and/or FA. No independent effects of atopic sensitization, total IgE levels, or AD severity (SCORAD) were observed on any of the platelet activation markers in either model (Table 2).

Here, increased plasma levels of platelet markers have been demonstrated to be associated with concomitant allergic disorders, in particular AR and FA. This observation indicates that

allergic inflammation affecting several organs and systems other than the skin is linked with enhanced release of multiple biologically active proteins from platelets that can modulate allergic immuno-inflammatory processes, for example platelet-derived growth factors [3,5].

In previous studies [1,6], β -TG and PF-4 were measured in allergic patients. A markedly enhanced skin expression of angiogenic VEGF, a potent cytokine leading to hyperpermeability of the vessel wall and endothelial cell proliferation, has been shown in AD [7]. The current large study expanded our knowledge on a role of platelets in allergic diseases by showing that AD, particularly when coexists with other allergic disorders, is potent enough to affect plasma VEGF levels and, thus, it might contribute to systemic effects produced by this cytokine. Furthermore, we determined PDGF, a potent regulator of fibrosis that, to the best of our knowledge, has not been measured in circulating blood of AD patients previously. Increased PDGF levels in circulating blood might be of importance given a role of PDGF and VEGF in fibrosis, the end result of chronic inflammatory reactions induced by various stimuli including allergic responses [8].

Tamagawa-Mineoka et al. [6] showed in 44 AD patients that plasma β -TG and PF-4 levels were correlated with AD severity (SCORAD) before the treatment with topical steroids, and were reduced after the therapy. We did not observe such an association in our representative real-life AD population. However, almost 90% patients in our study were classified as having mild-to-moderate AD, while in the former report most patients had severe AD [6]. It might be hypothesized that AD is specifically associated with increased platelet activation and its magnitude shows no consistent correlation with the disease severity.

It has been shown that AR alone, without concomitant AD, has no effect on circulating platelet markers [9]. In the present study, AR accompanying AD was a strong independent predictor of platelet activation markers. Similarly, FA was shown to be an independent predictor of β -TG, VEGF and PDGF. Therefore, it might be speculated that the presence of allergic skin inflammation observed in AD is potent enough not only to affect the levels of platelet markers per se, but also to enhance the platelet activation associated with inflammation of the upper respiratory tract as well as the gastrointestinal tract. However, we did not observe increased platelet activation in our AD patients with concomitant mild-to-moderate asthma, while previous studies reported increased platelet activation in asthmatic patients [1]. This discrepancy might partly be due to the fact that normal levels of platelet activation markers have been reported in patients with chronic asymptomatic asthma [10] and we excluded subjects with severe asthma.

Interestingly, no effect of atopic sensitization was observed on platelet activation markers, suggesting that atopic sensitization does not affect the activation of platelets observed in AD.

In conclusion, our results indicate that blood platelets are activated in AD patients. Increased platelet activation markers in AD patients are correlated with the presence of concomitant allergic disorders, especially AR.

Acknowledgment

The study was supported by a grant of Jagiellonian University (to A.U.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2011.07.001.

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Magdalena Nastalek

Department of Dermatology, Jagiellonian University Medical College,
Krakow, Poland

Daniel P. Potaczek

Center for Pediatrics, Clinic for Pediatric Pneumology, Allergology
and Neonatology, Hannover Medical School, Hannover, Germany

Anna Wojas-Pelc

Department of Dermatology, Jagiellonian University Medical College,
Krakow, Poland

Anetta Undas*

Institute of Cardiology, Jagiellonian University Medical College,
80 Pradnicka St., 31-202 Krakow, Poland

*Corresponding author. Tel.: +48 12 6143004; fax: +48 12 4233900
E-mail address: mmundas@cyf-kr.edu.pl (A. Undas)

11 May 2011

doi:10.1016/j.jdermsci.2011.07.001