

Expression of the Basophil-Specific Antibodies 2D7 and BB1 in Patients With Cutaneous Mastocytosis

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■ Abstract

Background: 2D7 and BB1 are thought to be basophil-specific markers. In this study, we tested both antibodies in different skin and mast cell disorders with the aim of determining whether it was possible to differentiate between benign and aggressive presentations of mastocytosis.

Methods: Using the antibodies 2D7, BB1, and c-Kit, we performed an immunohistochemical study of skin biopsy specimens from patients with cutaneous mastocytosis (15 urticaria pigmentosa and telangiectatic macularis eruptive perstans) and liver or bone marrow biopsy specimens from patients with systemic mastocytosis. A basophil leukemia cell line was used as a reference. Peripheral blood basophils from healthy donors were used as controls.

Results: We observed intense expression of 2D7 and BB1 in all skin biopsy specimens from patients with cutaneous mastocytosis. Immunostaining of liver and bone marrow specimens from patients with systemic mastocytosis with 2D7 and BB1 antibodies was negative. Specimens from patients with either type of mastocytosis showed similarly strong expression of c-Kit. The basophil cell line showed a 2D7 and a BB1 profile, with intense expression of c-Kit. Peripheral blood basophils exhibited notable immunostaining for 2D7, BB1, and c-Kit.

Conclusions: 2D7 and BB1 are expressed in cutaneous mastocytosis, although this expression is lost when mast cell proliferation is systemic, thus reflecting either a different cellular differentiation stage or the presence of basophils in these skin diseases.

Key words: 2D7. BB1. Mast cells. Basophils. Cutaneous mastocytosis. Systemic mastocytosis.

■ Resumen

Antecedentes: Los anticuerpos 2D7 y BB1 son marcadores específicos para basófilos. En el presente trabajo hemos estudiado el comportamiento de ambos marcadores en mastocitosis cutánea y sistémica, con el objeto de comprobar si las formas cutáneas benignas se podían diferenciar de las sistémicas.

Métodos: Hemos realizado inmunohistoquímica de biopsias de 15 pacientes con mastocitosis cutánea, empleando los anticuerpos c-Kit, 2D7 y BB1 y hemos comparado el resultado con biopsias hepáticas y de médula ósea de tres pacientes con mastocitosis sistémica frente a los mismos anticuerpos. Empleamos una línea celular de leucemia humana basofílica y basófilos aislados de un donante sano como controles.

Resultados: Hallamos un patrón 2D7 y BB1 intensamente positivo en todas las muestras de mastocitosis cutánea y basófilos de donantes sanos. Sin embargo, tanto el anticuerpo 2D7 como BB1 fue negativo en las muestras hepáticas y de médula ósea de mastocitosis sistémica así como en la línea celular de leucemia basofílica. C-Kit se expresó intensamente en todas las muestras.

Conclusión: 2D7 y BB1 se expresa en mastocitosis cutánea si bien se pierde cuando los mastocitos proliferan en su forma sistémica. Esto puede reflejar bien una diferenciación celular distinta o bien que los basófilos están presentes en las lesiones cutáneas de mastocitosis.

Palabras clave: 2D7. BB1. Mastocitos. Basófilos. Mastocitosis cutánea. Mastocitosis sistémica.

Introduction

Mastocytosis can be classified as cutaneous or systemic [1-3]. Although both types are mutually exclusive, it is unclear whether isolated urticaria pigmentosa could progress to systemic mastocytosis [4,5].

Several of the immunohistochemical markers used in the routine processing of tissue samples have been proposed for the identification of mast cells [6]. c-Kit is a well-known receptor that is routinely used to identify mast cells. The monoclonal antibodies 2D7 [7] and BB1 [8] can be used to identify basophils. 2D7 has a cytoplasmic and granular pattern and is located in secretory granules [7]. Its high sensitivity makes it reliable for the detection and quantification of basophils [9]. BB1 is a monoclonal antibody that recognizes basogranulin [10], a molecule of secondary granules. However, none of these basophil-specific markers can be identified using molecular techniques, and no information exists on their expression in the early differentiation of basophils and mast cells.

We investigated the expression of 2D7 and BB1 in various proliferative mast cell processes using c-Kit as a reference marker with the aim of determining whether it was possible to differentiate between cutaneous and systemic presentations of mastocytosis.

Methods

Samples

We tested 20 biopsy specimens from patients with urticaria pigmentosa and 6 liver or bone marrow biopsies from patients diagnosed with systemic mastocytosis. The human myeloid cell line KU-812F [11], which comprises immature basophils [12], was used as a reference. Basophils from 2 healthy donors were purified and used as controls. All slides were stained with Giemsa.

We followed the World Health Organization classification [13] to define each patient group. Urticaria pigmentosa was defined as comprising typical skin lesions, focal infiltrates of mast cells in the dermis, no evidence of systemic mastocytosis, and normal blood counts [13]. A telangiectatic subvariant (also referred to as telangiectasia macularis eruptiva perstans) was detected in 7 patients [3]. Three patients had systemic mastocytosis: patient 2 met 1 major and 2 minor criteria, whereas patient 3 had 1 major and 1 minor criterion. Patient 1 was classified as having indolent systemic mastocytosis, since mast cell aggregates were found in the bone marrow and liver biopsy specimens and no minor criteria were present [13]. The clinical characteristics of the patients are summarized in the Table.

Pellet Obtained From the Basophil Cell Line and Peripheral Blood Basophils

Immature basophils from the KU812F cell line were obtained from the Japan Collection of Research Bioresources. The cells were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine albumin, 2 mM L-glutamine, 10 mM hydroxyethyl piperazineethanesulfonic acid buffer, and antibiotics (100 U/mL penicillin G and 100 µg/mL streptomycin).

They were then cultured at 37°C in a humidified atmosphere with 5% CO₂ and passaged every 3-4 days.

Peripheral basophils from healthy donors and basophils from the KU812F line were centrifuged at 2000 rpm for 10 minutes, and the pellet was fixed in a volume of 1.5 mL of formol at 4% for 16-64 hours. The supernatant was discarded, and a second centrifugation was performed. The pellet was resuspended in 1 mL of 2% liquid agarose at 65°C. The solution was then centrifuged for 5 minutes at 1000 rpm to concentrate the cells in the agar. The agar-cell pellet was solidified at 4°C for at least 1 hour. Finally, the agar cone was carefully removed from the reaction tube with forceps, divided into 2 halves, wrapped in filter paper, and placed in a Tissue-Tek cassette [14].

Blood Basophil Cell Purification

Basophils from 2 healthy donors were purified by means of negative magnetic selection with an antibody cocktail (STEMCELL Technologies) following the manufacturer's instructions with >95% purity. Purity was assessed by means of flow cytometry (see below).

Testing of Paraffin-Embedded Samples Using Immunohistochemical Techniques

Specific basophil antibody 2D7 (IgG1) was kindly provided by LB Schwartz (Division of Rheumatology, Allergy and Immunology, Department of Internal Medicine, Virginia Commonwealth University, Richmond, Virginia, USA). Antigens were recovered at room temperature with pronase 0.5% for 10 minutes. The primary antibody was incubated at a 1:100 dilution overnight at room temperature.

Table. Clinical and Analytical Features of Patients Diagnosed With Systemic Mastocytosis

No.	Age	Sex	Skin Lesions	>15 MCs Aggregating in Bone Marrow Biopsy	>15 MCs Aggregating in Liver Biopsy	% of MCs in Bone Marrow Smear	Abnormal MC Morphology	c-Kit Mutation	Kit+MC Coexpress CD2 and/or CD25	Serum Trypsase Level
1	59	Male	Absent	Present	Present	35%	Absent	Absent	Absent	ND
2	66	Male	Absent	Present	Present	<25%	Present	Absent	ND	>200 µg/L
3	64	Male	Absent	Present	Present	<25%	Present	Absent	Absent	ND

Abbreviations: MC, mast cell; ND, not done.

An amplification method (Novocastra) was applied for 10 minutes. We then incubated the sample for 30 minutes with a polymer (a complex molecule with secondary antibody and streptavidin) from Novocastra. Diaminobenzidine was used as a chromogen. Samples were then counterstained with Harris hematoxylin. All samples were tested against c-Kit (Dako Diagnósticos SA) (1/100) and incubated for 1 hour at room temperature after antigen recovery by heating at pH7 in citrate buffer. We then incubated the samples for 30 minutes with the polymer from (Dako Diagnósticos SA). The slides were also counterstained with Harris hematoxylin. Positive and negative controls were obtained in each case. The controls consisted of small bowel tissue for c-Kit and peripheral blood basophils for BB1 and 2D7 antibodies.

All samples were tested against BB1 (kindly provided by A Walls, Immunopharmacology Group, Southampton General Hospital, Southampton, UK) after antigen recovery by heating at pH9. Samples were incubated at a 1:4 dilution overnight.

An amplification method (Novocastra) was applied for 10 minutes. The samples were then incubated for 30 minutes with the polymer from Novocastra. Diaminobenzidine was used as a chromogen. Slides were counterstained with Harris hematoxylin.

Double immunostaining Technique

Samples were treated with VECTABOND reagent, deparaffinized for 30 minutes, and immersed 3 times in absolute alcohol. We then blocked endogenous peroxidase in an H₂O₂ and methanol 3% solution for 30 minutes. The samples were hydrated at different concentrations of alcohol. Antigens were recovered by microwave in Tris/EDTA buffer at pH9 for 20 minutes. The unspecific background was blocked using normal goat serum diluted at 3% for 30 minutes at room temperature. The solution was incubated with the primary antibodies overnight at room temperature. A 1/200 dilution was used for c-Kit, and a 1/4 dilution was used for BB1.

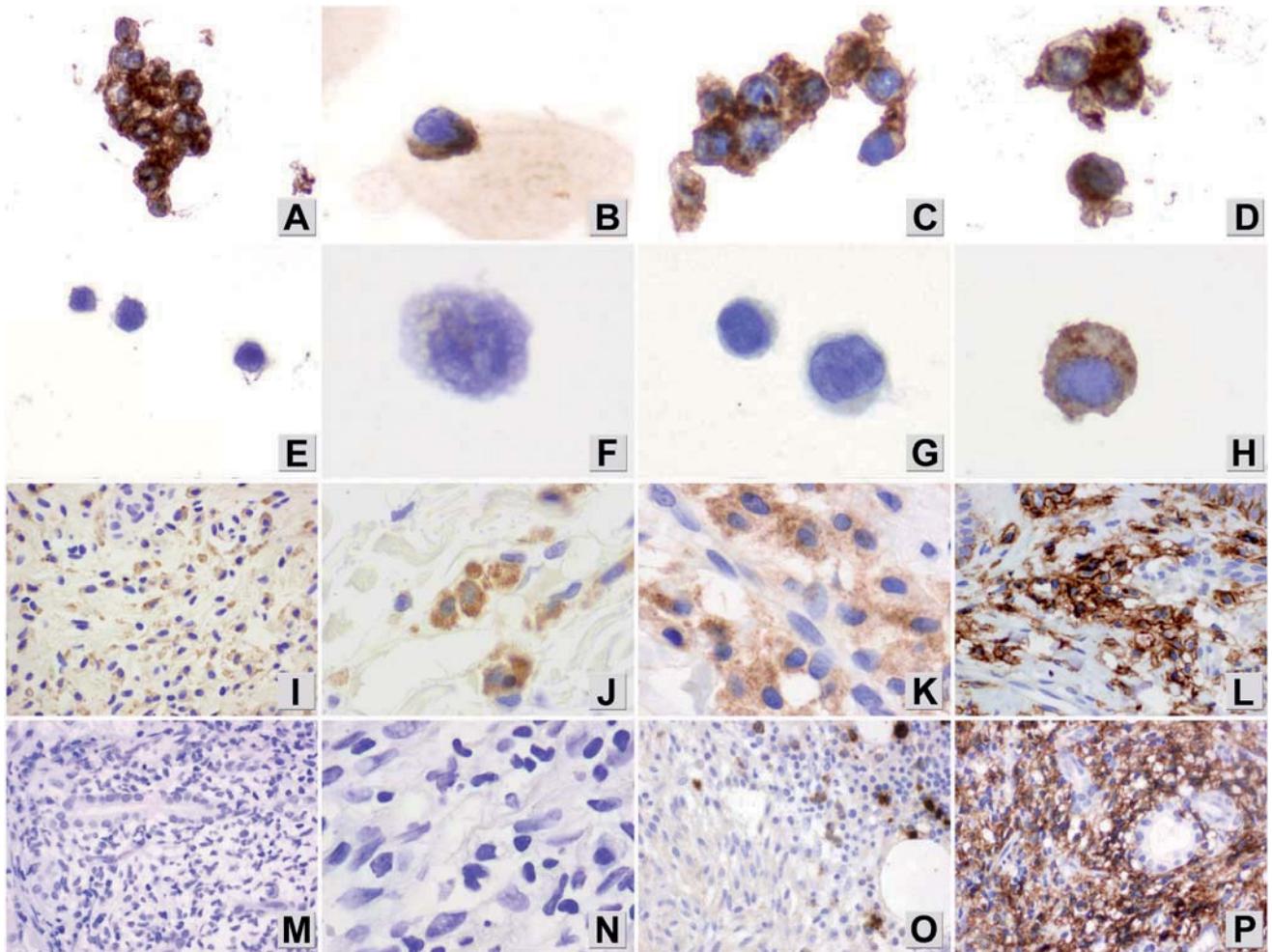


Figure 1. Peripheral blood basophils: Peripheral blood basophils showed intense immunostaining against 2D7 (A and B), BB1 (C), and c-Kit (D). Basophil cell line: The basophil cell line showed no immunoreactivity against 2D7 (E and F) or BB1 (G). However, intense cytoplasmic and granular immunoreactivity was observed against c-Kit (H). Urticaria pigmentosa: Intense expression of 2D7 (cytoplasmic and granular) was observed in the mast cells of all skin biopsy specimens from patients with urticaria pigmentosa (I and J) and BB1 (K). Intense immunoreactivity was also observed against c-Kit (L). Systemic mastocytosis: Neither liver nor bone marrow biopsy specimens from patients with systemic mastocytosis showed any immunostaining at all with 2D7 (M and N) or BB1 (O). In contrast, expression of c-Kit was very strong (P).

The samples were washed in Tris-buffered saline (TBS) and incubated with Alexa Fluor goat antirabbit 488 for 2 hours at room temperature. After immersion in TBS, a second incubation (2 hours at room temperature) was performed with the secondary biotinylated antigoat antibody at 1/200 in phosphate-buffered saline (PBS). This step was followed by incubation with horseradish peroxidase-conjugated streptavidin at a 1/500 dilution in PBS for 2 hours at room temperature. After additional washing in TBS, the solution was incubated with Alexa Fluor 568 tyramide at 1/500 in amplification buffer for 2 hours at room temperature. Finally, after further washing in TBS, the slides were mounted and studied using a confocal microscope (Zeiss LSM 510 Meta).

Flow Cytometry

Flow cytometry was performed as described elsewhere [15].

Results

Peripheral blood basophils exhibited intense immunostaining with 2D7, BB1, and c-Kit (Figure 1, A-D). Most KU812F cells stained positive with c-Kit (cytoplasmic and granular pattern) and negative with 2D7 or BB1 (Figure 1, E-H).

Urticaria pigmentosa specimens were strongly positive for 2D7, BB1, and c-Kit, with a cytoplasmic and granular pattern in a significant number of cells (Figure 1, I-L).

Liver and bone marrow biopsies from systemic mastocytosis specimens displayed no immunostaining at all with 2D7 or BB1. In contrast, expression of c-Kit was very strong (Figure 1, M-P).

Double immunostaining with c-Kit (green) and BB1 (red) in urticaria pigmentosa specimens revealed differing intensities for several of those stained with c-Kit. Immunostaining was more intense in large star-shaped cells with cytoplasmic spikes than in small round cells. However, only round cells showed BB1 antibody staining (Figure 2).

Discussion

2D7 and BB1 are useful for identifying basophils in tissue sections [7,8]. They are not expressed by mature mast cells. In order to investigate 2D7 and BB1 expression under mast cell-based conditions, we selected 2 diseases characterized by a high density of mast cells.

Staining for both 2D7 and BB1 was positive in all urticaria pigmentosa samples. Both antibodies have a cytoplasmic and granular pattern. When basophils are activated, they can be located in secretory granules [7,8], which are generally not metachromatic, although blood basophils with metachromatic granules have been reported in specific settings, eg, allergic diseases [16]. Metachromatic granules can be observed in mast cells after staining with Giemsa.

One of our most interesting findings is that both antibodies always behave in a similar way. It is noteworthy that expression of 2D7 and BB1 is lost in bone and liver biopsies from patients with proliferative mast cell disorders involving strong

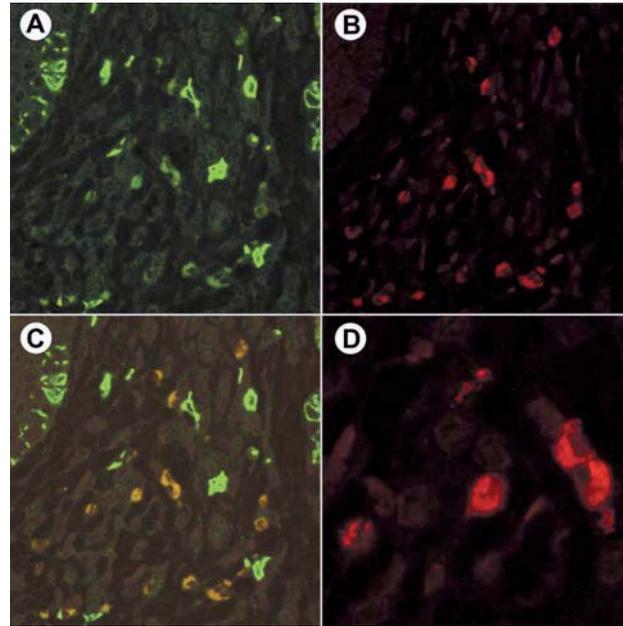


Figure 2. Results of double immunostaining in a representative case of cutaneous mastocytosis stained with both c-Kit (green) and BB1 (red). All cells stained positive with both antibodies, although with different intensities depending on the morphology.

A, Staining with c-Kit revealed large star-shaped cells (strong cytoplasmic expression) (original magnification, $\times 400$). B, With BB1, small round cells predominate (cytoplasmic expression) (original magnification, $\times 400$). C, Result of double immunostaining with BB1 and c-Kit using the appropriate filter (original magnification, $\times 400$). D, Detail of BB1-stained cells. Large cells with cytoplasmic expansions corresponding to mature mast cells did not stain with BB1.

expression in the benign counterpart of mastocytosis. Hence, the loss of 2D7 and BB1 expression is associated with the stage of maturation in mast cells. However, metachromatic granules are still present in these neoplastic mast cells. Likewise, it is worth mentioning that the loss of 2D7 and BB1 is also observed after staining of a human basophilic leukemia cell line, although contradictory findings have been reported [9,12]. Taken together, these observations seem to indicate that the loss of 2D7 and BB1 is associated with malignant neoplastic transformations of basophils or mast cells. The term mastocytosis covers a group of mast cell proliferative disorders (both local and systemic) with variable and sometimes unpredictable behavior. The myeloid cell line (KU812F) used in this study consists of immature basophils [11]. In their studies on human myeloid cell lines, Welker et al [12] concluded that induction of mast cells and basophils branches off at early and distinct points in myeloid cell development. We postulate that 2D7 and BB1 could be used as markers to differentiate local mastocytosis from systemic mastocytosis. However, more studies on mastocytosis are needed to confirm this promising insight.

The results of the double immunostaining study clearly show that, in cutaneous mastocytosis, all the cells of the tumoral infiltrate stain with c-Kit at different strengths.

Two types of cells and intermediate shapes are observed with c-Kit staining. The star-shaped cells generated by cytoplasmic extensions stained with a higher intensity than the small round cells. BB1, on the other hand, stains only the small round cells but not the large ones. The large c-Kit-positive cells had a mast cell phenotype, whereas the small round cells (both BB1- and c-Kit-positive) were similar to basophils. Thus, taking into account the specificity of BB1 for basophils reported elsewhere [3,4] and the selective immunostaining of c-Kit in mast cells, it could be hypothesized that the intermediate cells positive for both antibodies could belong to the same cell type in different states of activation.

The issue of c-Kit ligand expression by basophils remains open to debate. Some authors state that c-Kit ligand is not expressed by basophils [7] and propose c-Kit as a putative marker able to differentiate between basophils and mast cells. Other authors observed c-Kit receptors in activated basophils [16,17]. In our study, c-Kit was detected by immunohistochemistry in normal peripheral blood basophils from a healthy donor. Using flow cytometry, Kocabas et al [18] found that basophils from healthy volunteers express low levels of surface c-Kit. However, we should take into account that c-Kit might not have the biological significance in basophils that it has in mast cells, since a loss-of-function mutation in the c-Kit gene in rats had little effect on basophils, in contrast with mast cells.

To our knowledge, this is the first time that 2D7 and BB1 have been tested in urticaria pigmentosa, a benign cutaneous mast cell proliferative disorder.

In conclusion, according to our results, 2D7 and BB1 are expressed differently in urticaria pigmentosa and in systemic mastocytosis. Although both antibodies are highly recognized in cutaneous mastocytosis, they are not expressed in the systemic form. Thus, these antibodies could prove to be very useful markers. 2D7 and BB1 are also expressed in peripheral blood basophils; however, this expression disappears in immature basophils, as occurred in the human basophil leukemia cell line we tested. We also found expression of both antibodies in cutaneous mastocytosis samples, suggesting migration of basophils into skin lesions. Moreover, double immunostaining with BB1 and c-Kit makes it possible to distinguish between 2 different cell phenotypes and an intermediate one in biopsy specimens from patients with cutaneous mastocytosis. In contrast, c-Kit does not enable discrimination between immature basophils and mast cells, since immature basophils can express c-Kit.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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