

Enumeration and immunohistochemical characterisation of bone marrow basophils in myeloproliferative disorders using the basophil specific monoclonal antibody 2D7

H Agis, M-T Krauth, I Mosberger, L Müllauer, I Simonitsch-Klupp, L B Schwartz, D Printz, A Böhm, G Fritsch, H-P Horny, P Valent

J Clin Pathol 2006;**59**:396–402. doi: 10.1136/jcp.2005.029215

See end of article for authors' affiliations

Correspondence to:
Dr Peter Valent,
Department of Internal
Medicine I, Division of
Haematology and
Haemostaseology,
Medical University of
Vienna, Währinger Gürtel
18-20, A-1090 Vienna,
Austria; peter.valent@
meduniwien.ac.at

Accepted for publication
27 June 2005

Background: Basophils are highly specialised granulocytes that express a unique profile of antigens and increase in myeloproliferative disorders (MPD). In chronic myeloid leukaemia (CML), basophilia is a diagnostic and prognostic determinant. So far, however, no reliable approach for routine detection and enumeration of bone marrow basophils has become available.

Objective: To detect and enumerate basophils in bone marrow sections in patients with CML and other MPD

Methods: The anti-basophil antibody 2D7 was applied to paraffin embedded bone marrow sections from normal/reactive subjects (n = 31), patients with CML (chronic phase, n = 37; accelerated phase, n = 9), and other MPD (chronic idiopathic myelofibrosis (CIMF), n = 20; polycythaemia vera (PV), n = 20; essential thrombocythaemia (ET), n = 20; indolent systemic mastocytosis (ISM), n = 7).

Results: As assessed by serial section staining, 2D7⁺ cells were found to co-express myeloperoxidase, histidine decarboxylase, CD9, and CD43, but did not express B cell or T cell restricted antigens. 2D7⁺ bone marrow cells were found to increase in CML compared with normal/reactive bone marrow and other MPD (median numbers of 2D7⁺ cells/mm²: CML, 33; normal/reactive bone marrow, 6; CIMF, 10; PV, 6; ET, 5; ISM, 3; p < 0.05). The highest basophil counts were recorded in accelerated phase CML (115/mm²).

Conclusions: A novel immunohistochemical procedure has been established for basophil detection in normal bone marrow and MPD. This approach should help in the quantification of bone marrow basophils at diagnosis and during anti-leukaemic treatment.

Basophils are multifunctional haematopoietic cells that store histamine and other proinflammatory mediators in their granules.^{1–5} Under physiological conditions, basophils are primarily produced in the bone marrow.^{6–9} After maturation, they typically reside in the circulation until recruited into tissue sites of inflammation. Basophils express a unique composition of cytoplasmic mediators and cell surface membrane antigens.^{10–15} Although mast cells and basophils produce several mediators and surface antigens in common, they can clearly be discriminated from each other by their unique profile of granular enzymes and CD molecules.^{12–17} During the past two decades, the phenotype of human basophils has been analysed extensively using antibodies directed against various leucocyte differentiation antigens.^{14–18} Most of these studies have been performed on normal peripheral blood basophils.^{14–17} By contrast, little is known about the phenotype of bone marrow basophils.¹⁸

Basophilia is detectable in various haematopoietic neoplasms including chronic myeloid leukaemia (CML) and other myeloproliferative disorders (MPD).^{19–22} Especially in CML, basophilia is a typical finding.^{19–21} In addition, an increased percentage of basophils in the bone marrow and in the peripheral blood in CML is associated with a poor prognosis.^{23–29} Thus basophilia is an independent adverse prognostic variable in CML.^{27–29} Moreover, basophils typically increase in number in the bone marrow as well as in the blood during disease acceleration.^{23–27} Basophils originate from the malignant clone in patients with CML.^{30–31} However, whereas basophilia can readily be quantified in the peripheral blood and on bone marrow smears, no generally accepted and easily applicable staining protocol for the identification and

quantification of basophils in bone marrow biopsy sections has become available so far. In fact, basophils are difficult to identify by routine histochemistry, and no reliable immunohistochemical marker for basophil detection in the bone marrow has been described.

Recently, several basophil specific antibodies have been generated and used for the immunohistochemical detection of basophils in tissue sections.^{32–33} One of these granule associated antigens is recognised by a murine monoclonal antibody called 2D7.³³ In the present study, 2D7 was used to detect and enumerate basophils in bone marrow sections in patients with CML and other MPD. The results of our study show that 2D7 staining is a highly sensitive and reliable approach for the detection and quantification of bone marrow basophils in these patients.

METHODS

Bone marrow sampling and patients' characteristics

Bone marrow biopsy material (iliac crest) was examined in 46 patients with CML (chronic phase, n = 37; accelerated phase, n = 9), 20 with chronic idiopathic myelofibrosis (CIMF), 20 with polycythaemia vera, 20 with essential thrombocythaemia, seven with indolent systemic mastocytosis, and 31 control cases (normal/reactive marrow = no neoplasm involving the bone marrow). Diagnoses were established according to published criteria.^{34–35} The patients' characteristics are shown in table 1. Informed consent was

Abbreviations: CIMF, chronic idiopathic myelofibrosis; CML, chronic myeloid leukaemia; MPD, myeloproliferative disorder; VEGF, vascular endothelial growth factor

Table 1 Patient characteristics

| Diagnosis | Patients (n) | Median age (y) | F:M ratio | Median WBC ($\times 10^9/l$) | Blood basophils (median %) |
|-----------|--------------|----------------|-----------|--------------------------------|----------------------------|
| CML (all) | 46 | 57.5 | 1:2.1 | 110 | 5 |
| CML-CP | 37 | 57 | 1:2.1 | 103.5 | 4 |
| CML-AP | 9 | 58 | 1:2 | 258.4 | 18 |
| PV | 20 | 66.5 | 1:1.2 | 10.05 | 1 |
| ET | 20 | 58 | 1:1 | 9.5 | 2 |
| CIMF | 20 | 71 | 1:1.2 | 11.35 | 1 |
| ISM | 7 | 41 | 1:0.75 | 6.8 | 0 |

AP, accelerated phase; CIMF, chronic idiopathic myelofibrosis; CML, chronic myeloid leukaemia; CP, chronic phase; ET, essential thrombocythaemia; ISM, indolent systemic mastocytosis, PV, polycythaemia vera; y, years.

obtained in all cases before bone marrow or blood was obtained.

In 23 patients with CML, whole blood histamine levels were measured by a commercial radioimmunoassay (RIA) (Immunotech) as an indirect (objective) index of basophil lineage cells.

Antibodies

Various monoclonal and polyclonal antibodies were used to determine the phenotype of basophils in bone marrow sections. The monoclonal antibodies AB75 (CD2), PS1 (CD3), 1F6 (CD4), 4C7 (CD5), CD7-272 (CD7), 72F6 (CD9), 56C6 (CD8), 38C12 (CD13), 7 (CD14), 2H7 (CD16), FPC1 (CD22), 1B12 (CD23), 4C9 (CD25), and 1B6 (CD56) were purchased from Novocastra (Newcastle upon Tyne,

UK); C8/144B (CD8), L26 (CD20), 1F8 (CD21), BER-H2 (CD30), JC/70A (CD31), DF1485 (CD44), UCHL1 (CD45), PG-M1 (CD68), KP-1 (CD68R), JCB117 (CD79a), CR3/43 (anti-HLA-DR), and polyclonal antibodies against KIT (CD117) and myeloperoxidase were from Dako (Copenhagen, Denmark); the CD15 antibody DT07+BC97 from BioCare (Walnut Creek, California, USA); the monoclonal antibodies QBEND10 (CD34) and 97A6 (CD203c) from Immunotech (Marseille, France); MT1 (CD43) from Biotest (Frankfurt, Germany); and HNK-1 (CD57) from Becton Dickinson (San José, California, USA). The monoclonal antibodies B-B4 (CD138) and CCI (anti-chymase) were from SeroTec (Kidlington, Oxford, UK), a polyclonal anti-VEGF antibody from Santa Cruz (Santa Cruz, California, USA), the monoclonal antibody G3 (anti-tryptase) from Chemicon

Table 2 Specification of antibodies and staining techniques

| CD | Antibody (clone*) | Reactive antigen | Ig class | Source | Dilution | Retrieval technique† |
|-------|-------------------|------------------|----------|--------|----------|----------------------|
| CD2 | AB75 | LFA-2 | IgG1 | Mouse | 1:40 | Aut |
| CD3 | PS1 | TCR | IgG2a | Mouse | 1:50 | Aut |
| CD4 | 1F6 | T4 | IgG1 | Mouse | 1:10 | Aut |
| CD5 | 4C7 | gp67 | IgG1 | Mouse | 1:20 | Aut |
| CD7 | CD7-272 | gp40 | IgG1 | Mouse | 1:40 | MW |
| CD8 | C8/144B | T8 | IgG1 | Mouse | 1:20 | Aut |
| CD9 | 72F6 | MRP-1 | IgG1 | Mouse | 1:200 | MW |
| CD10 | 56C6 | CALLA | IgG1 | Mouse | 1:20 | Aut |
| CD13 | 38C12 | APN | IgG1 | Mouse | 1:80 | Aut |
| CD14 | 7 | LPS-R | IgG2a | Mouse | 1:100 | MW |
| CD15 | DT07+BC97 | LewisX | IgMκ | Mouse | 1:40 | MW |
| CD16 | 2H7 | FcγRIII | IgG2a | Mouse | 1:40 | Aut |
| CD20 | L26 | B1 | IgG2a | Mouse | 1:200 | MW |
| CD21 | 1F8 | C3dR | IgG1 | Mouse | 1:20 | Prot |
| CD22 | FPC1 | BL-CAM | IgG1 | Mouse | 1:40 | Aut |
| CD23 | 1B12 | FcεRII | IgG1 | Mouse | 1:40 | Aut |
| CD25 | 4C9 | IL-2Rα | IgG2b | Mouse | 1:40 | Aut |
| CD30 | BER-H2 | Ki-1 | IgG1 | Mouse | 1:40 | MW |
| CD31 | JC/70A | PECAM-1 | IgG1 | Mouse | 1:80 | MW |
| CD34 | QBEND10 | HPCA-1 | IgG1 | Mouse | 1:100 | MW |
| CD43 | MT1 | Leukosialin | IgG1 | Mouse | 1:400 | MW |
| CD44 | DF1485 | Pgp-1 | IgG1 | Mouse | 1:50 | MW |
| CD45 | UCHL1 | UCHL-1 | IgG2a | Mouse | 1:100 | MW |
| CD56 | 1B6 | NCAM | IgG1 | Mouse | 1:80 | Aut |
| CD57 | HNK-1 | HNK1 | IgM | Mouse | 1:10 | - |
| CD68 | PG-M1 | Macrosialin | IgG3 | Mouse | 1:100 | Prot |
| CD68R | KP-1 | Macrosialin | IgG1 | Mouse | 1:100 | Prot |
| CD79a | JCB117 | Igα/MB1 | IgG1 | Mouse | 1:25 | MW |
| CD117 | αKIT | KIT/SCFR | Poly | Rabbit | 1:150 | MW |
| CD138 | B-B4 | Syndecan1 | IgG1 | Mouse | 1:40 | MW |
| NC | CR3/43 | HLA-DR | IgG1 | Mouse | 1:200 | MW |
| NC | Polyclonal | MPO | Poly | Rabbit | 1:400 | MW |
| NC | Polyclonal | VEGF | Poly | Rabbit | 1:50 | MW |
| NC | Polyclonal | HDC | Poly | Rabbit | 1:1000 | MW |
| NC | G3 | Tryptase | IgG1 | Mouse | 1:5000 | - |
| NC | CCI | Chymase | IgG1 | Mouse | 1:1000 | Prot |
| NC | 2D7 | 2D7 | IgG1 | Mouse | 1:500 | Prot |

*Clone of monoclonal antibody.

†Retrieval techniques: Aut, autoclave; MW, microwave oven; Prot, proteinase type XXIV.

HPCA-1, haemopoietic progenitor cell antigen-1; IL2Rα, interleukin 2 receptor α; LFA-2, lymphocyte function associated antigen-2; NC, not yet clustered; SCF-R, stem cell factor receptor.

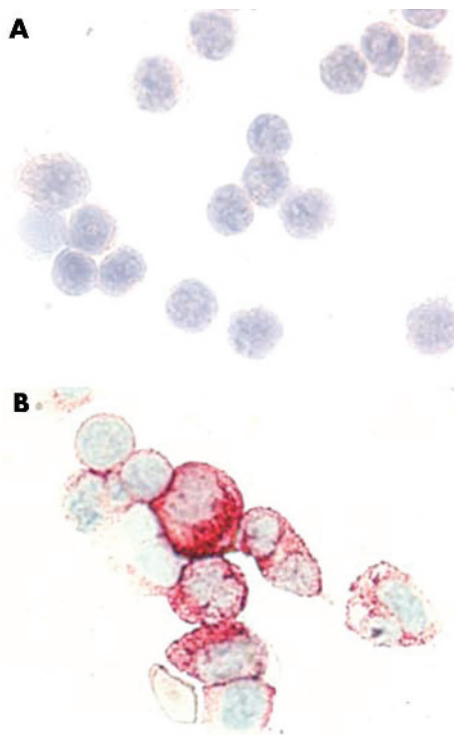


Figure 1 Specificity of 2D7 for basophils in chronic myeloid leukaemia (CML). Primary blood leucocytes obtained from a patient with CML were negatively sorted by flow cytometry using antibodies against CD203c (A) or an antibody against CD15 (B), and then were spun on cytospin slides. The basophil depleted (CD203c negative) cells did not react with the 2D7 antibody (A), whereas virtually all of the basophil enriched cells were labelled by 2D7 (B). Original magnification $\times 100$.

(Temecula, California, USA); and a polyclonal antibody against histidine decarboxylase from Progen (Heidelberg, Germany). The monoclonal antibody 2D7 was prepared as described.³³

Immunohistochemistry

Immunohistochemistry was performed on serial (2 μm) sections prepared from paraffin embedded, formalin fixed bone marrow specimens using the indirect immunoperoxidase staining technique as described previously.^{36–39} Endogenous peroxidase was blocked by methanol/ H_2O_2 . For basophil detection, the monoclonal antibody 2D7³³ was applied. Other monoclonal antibodies were used to confirm the presence of basophils and to determine their phenotype. A specification of monoclonal antibodies and the staining techniques applied are shown in table 2. In general, the antibodies were diluted in 0.05 M Tris-buffered saline (TBS, pH 7.5) plus 1% bovine serum albumin (BSA) (Sigma, St Louis, Missouri, USA). After washing, slides were incubated with biotinylated horse anti-mouse IgG or goat anti-rabbit IgG for 30 minutes, washed, and then exposed to avidin-biotin-peroxidase or streptavidin-biotin-peroxidase complex for 30 minutes. Diaminobenzidine hydrochloride (DAB) or 3-amino-9-ethylcarbazole (AEC) were used as chromogen. Slides were counterstained in Mayer's Haemalaun. Expression of antigens in basophils was determined by comparing cell reactivity in serial sections stained with 2D7 (every second consecutive section stained) and other antibodies.

Determination of basophil counts in bone marrow sections

The numbers of basophils in bone marrow sections were determined on 2D7 stained slides in all patients. Basophil

counting was done using an objective lens ($\times 20$) and an ocular lens ($\times 10$) containing an optical grid (original magnification $\times 200$). The ocular system was adapted to define a scanned area of 0.425 mm^2 . In each section, at least 10 areas were examined. The numbers of 2D7⁺ cells were expressed as cells/ mm^2 .

Separation of basophils by flow cytometry

In two CML donors with excessive basophilia (51% and 30% basophils in differential blood counts, respectively), peripheral blood leucocytes were separated by Ficoll and subjected to flow cytometry to enrich or to deplete basophils. For this purpose, cells were split and incubated either with the basophil specific monoclonal antibody 97A6 (CD203c) or with a monoclonal antibody against CD15, a broadly expressed myeloid antigen that usually is not detectable on basophils and therefore is widely used to enrich these cells by negative selection.^{10 16 17} Both cell fractions were subjected to flow cytometry and both populations were purified by cell sorting on a high speed sorter (FACSARIA, Becton Dickinson). After purification, cells were spun on cytospin slides and subjected to Wright Giemsa staining. CD15 negative cells (enriched basophils) were subjected to immunocytochemical staining using the 2D7 antibody as well as antibodies against CD9, CD13, CD15, CD25, CD68, CD68R, CD117, tryptase, chymase, myeloperoxidase, VEGF, and histidine decarboxylase. For comparison of antigen expression in primary CML cells, the CML derived basophil cell line KU812 was also stained with these antibodies. CD203c negative cells (basophil depleted cell fractions) were stained with monoclonal antibody 2D7 to reconfirm the specificity of 2D7 for basophils. Immunocytochemistry was undertaken as described previously.^{33 40}

Statistical analysis

To determine whether differences among basophil numbers between the various groups of patients were significant, standard statistical tests including the paired Student's *t* test and analysis of variance, were applied. Differences were considered to be significant when the *p* value was < 0.05 . To define the relations of basophil associated markers, linear correlations were applied and the correlation coefficients (*R*) determined.

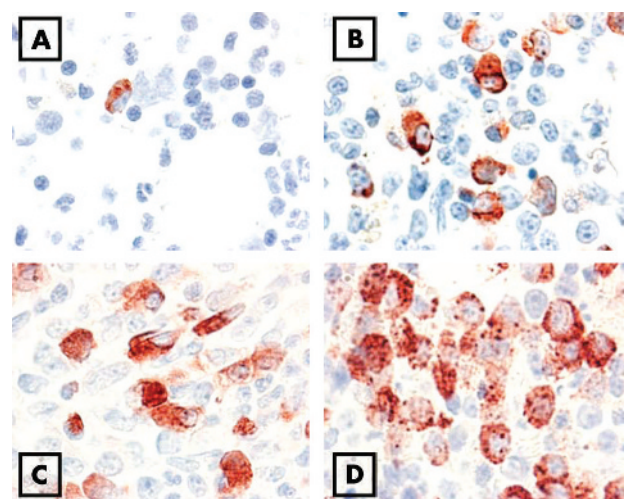


Figure 2 Immunohistochemical detection of basophils by 2D7 antibody in the bone marrow. Bone marrow sections were obtained from a patient without haematological neoplasm (control marrow) (A), one with chronic phase chronic myeloid leukaemia (CML) (B), one with accelerated phase CML (C), and one with accelerated CML and an excess of basophils (D). Slides were stained by the indirect immunoperoxidase technique.

Table 3 Expression of differentiation antigens in basophils

| Antigen | CD | Reactivity of antibodies with | | | Surface antigens phenotype* of | |
|-------------|-------|---------------------------------------|-----------------------------|------------|--------------------------------|-------|
| | | 2D7 ⁺ cells in CML bm, IHC | Isolated CML basophils, ICC | KU812, ICC | CML Basophils | KU812 |
| LFA-2 | CD2 | - | NT | NT | - | - |
| TCR | CD3 | - | NT | NT | - | - |
| T4 | CD4 | +/- | NT | NT | -† | +/- |
| gp67 | CD5 | - | NT | NT | - | - |
| gp40 | CD7 | - | NT | NT | - | - |
| T8 | CD8 | - | NT | NT | - | - |
| MRP-1 | CD9 | + | + | + | + | + |
| CALLA | CD10 | - | NT | NT | - | - |
| APN | CD13 | + | + | + | + | + |
| LPS-R | CD14 | - | - | - | - | - |
| LewisX | CD15 | + | +/- | + | - | - |
| FcγRIII | CD16 | - | NT | NT | - | - |
| B1 | CD20 | - | NT | NT | - | - |
| C3dR | CD21 | - | NT | NT | - | - |
| BL-CAM | CD22 | - | NT | NT | - | - |
| FcεRII | CD23 | - | NT | NT | - | - |
| IL-2Rα | CD25 | +/- | + | + | + | + |
| Ki-1 | CD30 | - | NT | NT | - | - |
| PECAM-1 | CD31 | +/- | + | + | + | + |
| HPCA-1 | CD34 | - | - | - | - | - |
| Leukosialin | CD43 | + | NT | NT | + | + |
| Pgp-1 | CD44 | + | + | + | + | + |
| UCLH-1 | CD45 | + | NT | NT | + | + |
| NCAM | CD56 | - | NT | NT | - | - |
| HNK1 | CD57 | - | NT | NT | - | - |
| Macrosialin | CD68 | + | +/- | + | NT | NT |
| Macrosialin | CD68R | + | + | + | NT | NT |
| Igα/MB1 | CD79α | - | NT | NT | - | - |
| KIT/SCFR | CD117 | +/- | +/- | +/- | - | +/- |
| Syndecan1 | CD138 | - | NT | NT | - | - |
| HLA-DR | NC | +/- | +/- | +/- | -† | +/- |
| MPO | NC | + | + | + | NT | NT |
| VEGF | NC | + | + | + | NT | NT |
| HDC | NC | + | + | + | NT | NT |
| Tryptase | NC | +/- | + | + | NT | NT |
| Chymase | NC | - | - | NT | NT | NT |

Score of percentage of 2D7⁺ cells that were labelled: +, 50–100% of all cells reactive; +/-, 10–49% cells reactive; -, <10% of all cells stained.

*Data refer to published results.¹⁴⁻¹⁶

†After short term culture, CML basophils express surface CD4 and surface HLA-DR.¹⁰

bm, bone marrow; CML, chronic myeloid leukaemia; ICC, immunocytochemistry; IHC, immunohistochemistry; NC, not yet clustered; NT, not tested.

RESULTS

Demonstration that 2D7⁺ CML cells are basophils

In a first step, we reconfirmed the specificity of 2D7 using Ficoll separated primary peripheral blood leucocytes obtained from patients with CML showing basophilia. These cells were subjected to flow cytometry to obtain a CD203c negative (basophil depleted) fraction and a CD15 negative (basophil enriched) fraction. As expected, the CD203c negative fraction contained fewer than 1% basophils whereas the CD15 negative fractions contained >80% basophils. The monoclonal antibody 2D7 was found to react with enriched CML basophils, but did not react with bone marrow cells depleted of basophils (CD203c negative cells) (fig 1). Identical results were obtained in two CML donors. These data suggest that 2D7 is a basophil specific antigen in patients with CML but is not expressed in other leucocytes, confirming result obtained with normal blood basophils.³³

Immunophenotypic characterisation of bone marrow basophils in CML

In a next step, we established the immunophenotype of leukaemic basophils using serial bone marrow sections obtained from patients with CML. Using 2D7 antibody, basophils were easily detectable in all patients, although the numbers varied among donors (fig 2). As assessed by serial section staining, the 2D7⁺ CML basophils were found to co-express myeloperoxidase, tryptase, and histidine decarboxylase. In addition, basophils expressed CD9, CD13, CD31,

CD43, CD45, and CD68. By contrast, basophils did not react with monoclonal antibodies against CD2, CD3, CD5, CD7, CD8, CD10, CD14, CD16, CD20, CD21, CD22, CD23, CD30, CD56, CD57, CD79a, or CD138 (table 3). This phenotype corresponds to the well established surface marker profile of blood basophils.^{10 14 15 34} An interesting finding was that basophils express low levels of CD15, a marker antigen that is usually not detectable on the surface of normal or CML derived basophils unless these cells are exposed to neuraminidase.^{10 14 15}

The phenotype of basophils could also be confirmed by immunocytochemistry using enriched CML basophils (sorted CD15 negative cells) and KU812 cells spun on cytospin slides. In line with the data obtained by immunohistochemistry, CML basophils and KU812 cells were found to react with antibodies against 2D7 (fig 1) and myeloperoxidase (fig 3), as well as with antibodies against CD9, CD13, CD15, CD25, CD31, CD44, CD68, histidine decarboxylase, VEGF, and tryptase. A summary of all staining results obtained with CML basophils and KU812 cells is shown in table 3.

Numbers of 2D7⁺ cells in patients with various myeloproliferative disorders

The numbers of 2D7⁺ cells (basophils) were determined in bone marrow sections in patients with CML, patients with other MPD, and in normal/reactive bone marrow. In normal/reactive bone marrow, the number of 2D7⁺ (mean (SD)) was 8 (5) cells/mm² (median 6; range 0 to 18) (fig 2A). In patients

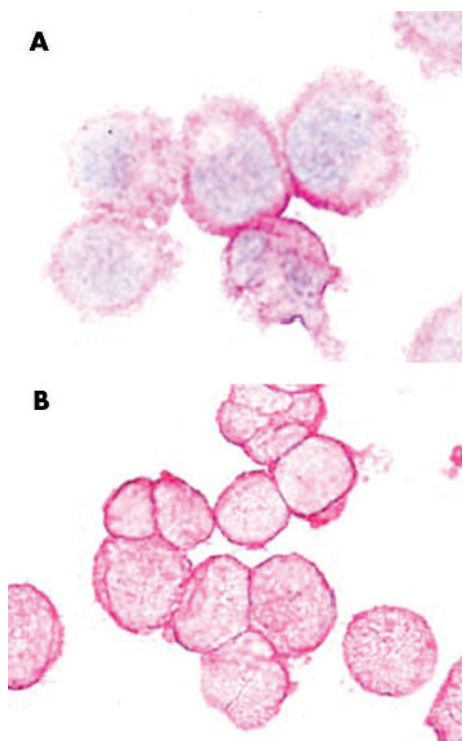


Figure 3 Immunocytochemical detection of myeloperoxidase in chronic myeloid leukaemia (CML) basophils. The CML derived basophil cell line KU812 (A) and enriched FACS sorted primary CML basophils (B) were spun on cytospin slides and stained with an antibody against myeloperoxidase (MPO). Both types of cells reacted with the anti-MPO antibody. The antibody did not label lymphocytes (not shown).

with CML, larger numbers of 2D7⁺ cells (basophils) were found (all CML patients: mean 46 (44) cells/mm², median 33, range 2 to 211; $p < 0.05$; CML-CP: mean 26 (14) cells/mm², median 28, range 2 to 57, $p < 0.05$) (fig 2, panels B, C, and D, and fig 4). The number of 2D7⁺ cells in patients with CIMF was 13 (12) cells/mm² (median 10; range 2 to 45). In patients with polycythaemia vera, the number of 2D7⁺ cells was 7 (5) cells/mm² (median 6; range 1 to 19), in essential thrombocythaemia 6 (4) (median: 5; range: 1–15 cells/mm²), and in indolent systemic mastocytosis, the number of 2D7⁺ cells was 5 (4) cells/mm² (median 3; range 2 to 14). Figure 4 shows the numbers of 2D7⁺ cells (mean and SD) in all groups of patients. The largest numbers of 2D7⁺ cells (basophils) were found in patients with accelerated phase CML (mean 126 (33) cells/mm² (median 115, range 103 to 211; $p < 0.05$) (fig 2, panels C and D, and fig 4). Figure 2D shows 2D7⁺ cells from a patient with accelerated phase CML in whom a massive increase in basophils was found. In this patient, the final diagnosis was “secondary basophilic leukaemia”.

Correlation between 2D7⁺ cells and other haematological indices in CML

In patients with CML, the (median) number of 2D7⁺ cells was correlated with various haematological indices including white blood count, peripheral blood basophils, and whole blood histamine levels. In these analyses, significant correlations were found between the numbers of 2D7⁺ bone marrow cells and numbers of peripheral blood basophils (fig 5A), and between the numbers of 2D7⁺ cells and the total blood histamine levels (fig 5B). By contrast, there was no correlation between 2D7⁺ bone marrow cells and peripheral blood leucocytes (fig 5C). As expected, a highly significant correlation between the numbers of peripheral blood

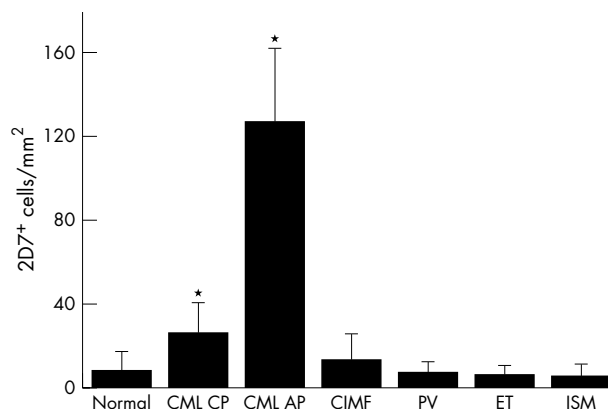


Figure 4 Numbers of 2D7⁺ cells in the bone marrow of patients with MPD. Numbers of 2D7⁺ cells in the bone marrow sections were determined by morphometry using an adapted microscope (see text). The figure shows the mean numbers of 2D7⁺ cells (basophils) per mm² in each group of patients. Results represent the mean and SD of all donors examined in each group. * $p < 0.05$ v normal bone marrow.

basophils and whole blood histamine levels was found (fig 5D). Interestingly, in patients with other MPD, the correlation between 2D7⁺ bone marrow cells and peripheral blood basophils (and the other variables analysed) was less pronounced than the correlations obtained in patients with CML (not shown).

DISCUSSION

Basophilia is an important feature of MPD, often a diagnostic finding, and sometimes even of prognostic significance.^{20 22–28} However, so far no reliable immunohistochemical marker for basophil detection and enumeration in MPD has become available. We have established 2D7 as a powerful new marker for the immunohistochemical analysis of basophils in patients with MPD. The results of our study show that 2D7 is specific for basophils in normal bone marrow as well as bone marrow in patients with MPD, and that the numbers of basophils can easily be quantified (or estimated) in 2D7 stained bone marrow sections. The 2D7 antibody should therefore be considered a novel helpful marker for basophil detection in haematopathology.

Although the 2D7 antibody is known to label basophils specifically in inflamed tissues^{41–43} and in normal peripheral blood,³³ it was of pivotal importance to reconfirm the specificity of 2D7 for basophils in patients with MPD. To address this issue, two different approaches were used. First, purified CML basophils and CML cells depleted of basophils, were analysed. In these experiments, we were able to show that 2D7 is selectively expressed in CML basophils, but is not expressed in basophil depleted cell fractions. In consecutive experiments, serial section staining was carried out in order to reconfirm that the 2D7 stained bone marrow cells express the established phenotype of human basophils.^{14–16}

Various previous studies have shown that basophils express a unique cell surface marker profile, including myeloid determinants as well as activation linked cell surface antigens.^{10 11 14–16 34 44} This phenotype has been described for normal blood basophils and for CML derived basophils.^{10 14–16} However, no systematic immunohistochemical analysis of bone marrow basophils has been presented so far. We now show that 2D7⁺ basophils in the bone marrow in patients with CML express a unique phenotype which corresponds largely to the cell surface phenotype of (normal and CML) basophils. However, some of the antigens detected in CML basophils by immunohistochemistry, like CD4 or CD15, are usually not detectable on the surface of normal blood

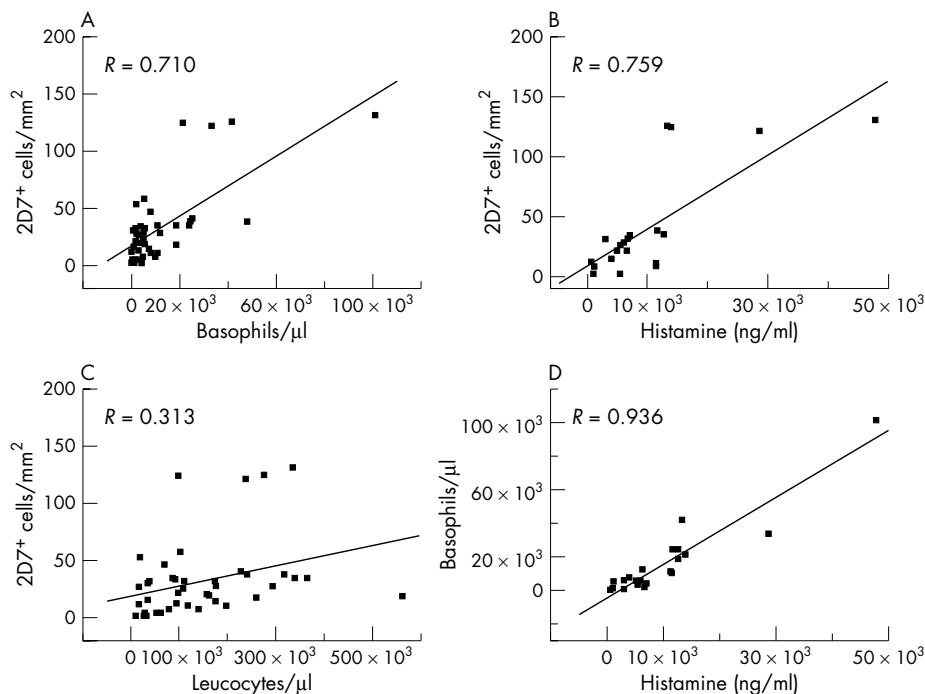


Figure 5 Correlation between the numbers of 2D7⁺ cells and other indices in chronic myeloid leukaemia (CML). Correlations were determined between the median numbers of 2D7⁺ cells in bone marrow sections (2D7⁺ cells per mm²) and absolute numbers of peripheral blood basophils (calculated from total leucocyte counts and the percentage of basophils in differential counts) (A), between the numbers of 2D7⁺ bone marrow cells and whole blood histamine levels (measured by radioimmunoassay) (B), between the numbers of 2D7⁺ bone marrow cells and blood leucocytes (C), and between absolute numbers of blood basophils and whole blood histamine levels (D). The correlation coefficient (R) is shown in each case. All correlations except that between the numbers of 2D7⁺ bone marrow cells and leucocytes were statistically significant ($p < 0.05$).

basophils.^{10 14–16 34} With regard to CD15, this antigen is indeed expressed on the surface of basophils, but is masked by gangliosides and is thus detectable only after treatment with neuraminidase.¹⁰ In the case of CD4, the discrepancy may have several explanations. First, the antibody used may cross react with other antigens or may show non-specific binding to basophils. An alternative explanation may be that CML basophils express very small amounts of CD4 on their cell surface. In this regard it is noteworthy that CML basophils can react with CD4 antibodies in cell surface staining experiments when these cells are kept in culture for several days.¹⁰ Whether CML basophils indeed express functional CD4 and thereby can interact with class II HLA antigen or the HIV virus remains presently unknown.

In CML, the numbers of basophils are almost invariably increased and correspond to the phase of disease.^{20 22 23–45} Especially in the accelerated phase of CML, many patients present with highly upregulated numbers of basophils.^{22–26 45} This phenomenon was confirmed in the present study by 2D7 staining. In fact, the largest numbers of 2D7⁺ cells were recorded in patients with accelerated phase CML. Thus the 2D7 antibody is a reliable marker and helpful to enumerate basophil counts in various phases of CML. In this regard, it is noteworthy that disease acceleration in CML is even defined by bone marrow basophilia.^{28 35 36} In addition, 2D7 may be helpful for monitoring of basophils—that is, their decrease during the first months of treatment with tyrosine kinase inhibitors.

In a few CML patients, basophilia is excessive and results in the clinical (pathological) picture of (secondary) basophilic leukaemia.^{37 38} Such excessive basophilia was also found in one of the patients examined in the current study. In this particular patient, more than 30% of all nucleated bone marrow cells appeared to react with the 2D7 monoclonal antibody. Thus the 2D7 antibody may also be

a helpful marker to confirm the presence of basophilic leukaemia.

Basophils are increased not only in CML, but also in most other subtypes of MPD.^{19 21 22} Therefore we extended our analyses using 2D7 monoclonal antibody to various groups of patients with MPD. However, interestingly, the numbers of 2D7⁺ bone marrow basophils were found to be in the same range in patients with other MPD compared with normal/reactive bone marrow. An interesting finding was that bone marrow basophils are not increased in patients with indolent systemic mastocytosis. In these patients, the mast cell infiltrates were clearly composed of 2D7[−] cells, and the remaining normal appearing marrow did not contain increased numbers of 2D7⁺ cells when compared with normal/reactive bone marrow. This observation provides further evidence that basophils and mast cells represent two different haematopoietic cell lineages.¹⁵ No evidence for the previously reported basophil/mast cell hybrids⁴⁶ was found in the current study, in agreement with the observations of Foster *et al.*⁴⁷

An important question addressed in our study was whether the numbers of 2D7⁺ bone marrow cells correlate with other basophil related or disease related indices. Indeed, the numbers of bone marrow basophils did correlate with the numbers (percentages) of peripheral blood basophils, histamine levels, and to a lesser degree, the leucocyte counts. These findings further document the value of the 2D7 monoclonal antibody in the immunohistochemical quantification of basophilia in patients with MPD. On the other hand, we quantified 2D7⁺ cells on a “per mm²” basis, so a possible influence of variation in cellularity between patients and disease categories needs to be taken into account. However, the cellularity among MPD patients did not differ extensively, so that such an influence may not change the overall message of this report. In this regard it should also be

pointed out that in the non-CML MPDs, the numbers of 2D7⁺ cells did not vary significantly from each other and not much from controls, so that the stain may not be helpful in separating non-CML MPD from reactive basophilia.

In summary, we have established a novel immunohistochemical staining technique for the detection and enumeration of basophils in routinely processed bone marrow trephine biopsy sections. This may be a helpful new approach in haematopathology.

ACKNOWLEDGEMENTS

We wish to thank Ilona Schliephake, Agnes Dummer, Mariola Erdmann-Jensko, Tanja Oeltermann, Katharina Vogel, and Christoph Kroll (University of Lübeck) as well as Susanne Herndlhofer and Hans Semper (Medical University of Vienna, Department of Internal Medicine I) for skilful technical assistance. This study was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung in Österreich (FWF) grant No P17205-B14, FWF grant No SFB-018/09, FWF-Charlotte Bühler grant No H185-B04, as well as grant No AI20487 from the National Institutes of Health, Bethesda.

Authors' affiliations

H Agis, M-T Krauth, A Böhm, P Valent, Department of Internal Medicine I, Division of Haematology and Haemostaseology, Medical University of Vienna, Austria

I Mosberger, L Müllauer, I Simonitsch-Klupp, Department of Clinical Pathology, Medical University of Vienna

L B Schwartz, Department of Internal Medicine, Division of Rheumatology, Allergy and Immunology, Virginia Commonwealth University, Richmond, Virginia, USA

D Printz, G Fritsch, Children's Cancer Research Institute, St Anna Children's Hospital, Vienna, Austria

H-P Horny, Department of Pathology, University of Lübeck, Germany

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