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# Clinicopathological features and prognostic significance of CXCL12 in blastic plasmacytoid dendritic cell neoplasm

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***Background:*** Blastic plasmacytoid dendritic cell neoplasm (BPDC) is a rare hematologic neoplasm, which almost always involves the skin and shows poor prognosis.

***Objective:*** The aim of our study was to enhance BPDC diagnosis and indications for prognosis.

***Methods:*** This study involved 26 patients with BPDC. To investigate the histogenesis of BPDC, we reviewed the clinical features and stained markers of various hematopoietic lineages, chemokines, and their receptors.

***Results:*** Bone-marrow infiltration was detected in 13 of the 19 cases examined and leukemic changes in 18. Complete remission was achieved in 14 cases, but more than half of the patients showed recurrence within a short time, and 14 patients died of the disease after 1 to 25 months (mean 8.5 months). Positivity for CD123 was detected in 18 of 24 cases and for T-cell leukemia 1 in 18 of 22 cases. Of the chemokines and their receptors, 8 of 15 skin biopsy specimens proved to be positive for CXCL12. Leukemic change subsequent to skin lesions occurred in 7 of 8 CXCL12-positive cases (87.5%) and in 3 of 6 CXCL12-negative cases (50%). Seven of the 8 CXCL12-positive patients (87.5%) and two of the 6 CXCL12-negative patients (33.3%) have died, whereas one of 8 CXCL12-positive patients (12.5%) and 4 of 6 CXCL12-negative patients (66.7%) remain alive.

***Limitations:*** The number of patients was limited.

***Conclusions:*** We speculate that the presence of CXCL12-positive cells in the skin may be associated with leukemic change and a poor prognosis. (J Am Acad Dermatol 2012;66:278-91.)

***Key words:*** blastic plasmacytoid dendritic cell neoplasm; CD123; chemokine; chemokine receptor; CXCL12; CXCR4; T-cell leukemia 1.

Blastic plasmacytoid dendritic cell neoplasm (BPDC) is a rare hematopoietic tumor characterized by frequent skin involvement, a rapid aggressive course. and poor prognosis despite good initial response to cytostatic therapy.[1-7] During the course of the disease, the typical presentation at the time of diagnosis consists of isolated cutaneous lesions, followed by systemic dissemination, including involvement of peripheral blood, bone marrow, lymph nodes, and other tissues.[8,9]

Discovery of BPDC was followed by the notion that BPDC was of CD56$^+$ natural killer (NK) lineage, but it has since been recognized that these leukemic cells shared the same characteristics as plasmacytoid dendritic cells (pDC).[1-3,6,10,11] Now this neoplasm is thought to be derived from the pDC lineage.[1,2,9] In

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Funding sources: None.
Conflicts of interest: None declared.
Accepted for publication December 22, 2010.
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Published online August 12, 2011.
0190-9622/$36.00
© 2011 by the American Academy of Dermatology, Inc.
doi:10.1016/j.jaad.2010.12.043

the current World Health Organization (WHO) classification, the diagnostic term "BPDC" is suggested for tumors satisfying the diagnostic criteria for this series and BPDC is categorized as acute myeloid leukemia (AML) and related precursor neoplasms.[12]

Chemokine receptors mediate the migration of lymphocytes through binding of their ligands. Besides their functions in the immune system, these receptors also play a critical role in tumor initiation, promotion, and progression. Numerous studies have established an association between lymphoma/leukemia and chemoattractants. For example, CXCL12 enhances migration of follicular lymphoma cells,[13] and the circuitry of CXCL12 and CXCR4 (a CXCL12 receptor) appears to be crucial for migration of chronic lymphocytic leukemia[14,15] and acute lymphoblastic leukemia (ALL) B cells.[16] CXCR4 overexpression was found to indicate a poor prognosis for patients with AML.[17] T-cell large granular lymphocytic leukemia cells mainly express CXCR1 and CXCR2, whereas peripheral T-cell lymphoma cells usually express CCR4, CCR6, and CCR7.[18] CXCR3 was reported to be present in marginal zone lymphoma.[19] However, chemoattractants that stimulate the locomotion of BPDC are insufficiently known.

**CAPSULE SUMMARY**

- Blastic plasmacytoid dendritic cell is an aggressive tumor with a generally poor prognosis.
- Patients with CXCL12-positive blastic plasmacytoid dendritic cell are at a higher risk of death than those with CXCL12-negative disease.
- Allogeneic stem cell transplantation should be considered as first-line treatment for cases of blastic plasmacytoid dendritic cell exhibiting high CXCL12 expression.

The aims of our study were to review the clinical, histologic, and immunophenotypic features of BPDC diagnosis and to investigate the chemokine expression patterns and potential influence on prognosis.

## METHODS

### Biologic materials

We obtained samples from 26 Japanese patients with BPDC diagnosed at the Department of Pathology, Kurume University, Japan, between 2004 and 2009. Skin, lymph node, or bone-marrow biopsies or aspirates were performed after obtaining informed consent from all patients or their guardians. Paraffin-embedded tissues of almost all patients were available for examination and frozen tissues and cell suspensions for the remaining patients. Histopathological diagnoses were based on the new WHO classification and carried out by 3 pathologists (K. H., Y. K., and K. O.). Clinical information was obtained by reviewing the tumor registry records and/or the patients' medical charts.

### Immunohistochemistry

The paraffin-embedded specimens were used for immunohistochemical analysis of CD4 (Medical & Biological Laboratories Co Ltd, Nagoya, Japan), CD56 (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom), CD123 (Pharmingen, San Diego, CA), CD68 (KP-1) (DakoCytomation, Glostrup, Denmark), CD99 (DakoCytomation), CD3 (Novocastra Laboratories), CD8 (Novocastra Laboratories), myeloperoxidase (MPO) (DakoCytomation), terminal deoxynucleotidyl transferase (Supertec, Bethesda, MD), T-cell intracellular antigen-1 (Immunotech, Marseille, France), CD34 (Beckman Coulter, Tokyo, Japan), CD117 (c-kit) (DakoCytomation), CD1a (Immunotech), GranzymeB (Novocastra Laboratories), Langerin (Abcam, Cambridge, United Kingdom), MT-1 (CD43) (eBioscience, San Diego, CA), lysozome (Denver Biomedical Inc, Golden, CO), S-100 (DakoCytomation), MIB-1 (DakoCytomation), CD20 (L26) (DakoCytomation), CD45RO (UCHL-1) (DakoCytomation), cutaneous lymphocyte-associated antigen (CLA) (Ancell Cor, Bayport, MN), T-cell leukemia 1 (TCL1) (Upstate Biotechnology Inc, Lake Placid, NY), CXCR3 (Pharmingen), CXCL9 (R&D Systems, Minneapolis, MN), CXCR4 (R&D Systems), CXCL12 (R&D Systems), CCR5 (DakoCytomation), CCL5 (Pharmingen), CCL2 (R&D Systems), CCR7 (Medical & Biological Laboratories Co Ltd), CCR4 (Pharmingen), and CXCL10 (R&D Systems). Heat-mediated antigen retrieval was used for all analyses except those for CLA and CXCR4. CLA was detected with HECA-452, a monoclonal antibody, kindly provided by Prof T. Yoshino of Okayama University. Frozen sections were used for staining with the respective antibodies against CD13 (Beckman Coulter), CD14 (Pharmingen), CD33 (Beckman Coulter), T-cell receptors (TCR)$\gamma/\delta$, and TCR$\beta$F1. Immunohistochemical studies were performed manually on paraffin-embedded material and on cryostat-cut sections as previously described.[20] Appropriate positive and negative control experiments were run simultaneously. To detect Epstein-Barr virus (EBV)-encoded nuclear small nonpolyadenylated RNA, each paraffin section was subjected to EBV in situ hybridization using a 30-base oligonucleotide that was complementary to a portion

*Abbreviations used:*

| | |
|---|---|
| ALL: | acute lymphoblastic leukemia |
| AML: | acute myeloid leukemia |
| BPDC: | blastic plasmacytoid dendritic cell neoplasm |
| CLA: | cutaneous lymphocyte-associated antigen |
| EBV: | Epstein-Barr virus |
| FITC: | fluorescein isothiocyanate |
| MPO: | myeloperoxidase |
| NK: | natural killer |
| pDC: | plasmacytoid dendritic cells |
| PE: | phycoerythrin |
| TCL1: | T-cell leukemia 1 |
| TCR: | T-cell receptors |
| WHO: | World Health Organization |

of the *EBER1* gene, as previously described.[21] The staining results were evaluated semiquantitatively by two independent observers and cases were scored as positive if more than 30% of the tumor cells were positive. As explained elsewhere, immunostaining was considered negative if less than 10% of the tumor cells failed to stain.

### Flow cytometry

Cell suspensions from 17 patients with BPDC (skin, $n$ = 7; lymph node, $n$ = 5; and bone marrow, $n$ = 5) were prepared for flow cytometry. Flow cytometry was performed with a flow cytometer (FACSCalive, Becton-Dickinson, Mountain View, CA) and the Cell Quest software program (Becton-Dickinson) using conventional methods described previously.[22] Briefly, cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies by using the following combinations: CD2 (FITC), CD3 (FITC), CD4 (FITC), CD5 (PE), CD7 (PE), CD8 (PE), CD10 (PE), CD11c (PE), CD16 (FITC), CD19 (PE), CD20 (FITC), CD25 (PE), CD30 (FITC), CD34 (FITC), and CD56 (PE). CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD16, CD19, CD34, and CD56 were obtained from Coulter Clone (Hialeah, FL); CD11c, CD20, and CD25 were obtained from Becton-Dickinson; and CD30 from DakoCytomation.

### DNA analysis

The remainder of the frozen tissue was used for DNA isolation and gene analysis. Before DNA analysis, the samples were confirmed to contain lymphoma cells by means of hematoxylin-eosin and immunohistochemical staining of the frozen samples. Skin and lymph node specimens (skin, $n$ = 3 and lymph node, $n$ = 4) were subjected to conventional cytogenetic studies with G-banding techniques. For the next step, polymerase chain reaction was performed on DNA of 8 cases isolated from frozen material by using fluorescent primers for analysis of TCR. The samples were examined for TCR genes $C\beta$ and/or $J\delta$.

### Statistical analysis

Survival analysis was performed with the Kaplan-Meier method and survival curves were compared by means of the Wilcoxon test, whereas the relationship between prognosis and survival was evaluated by univariate $\chi^2$ analysis combined with the Wilcoxon tests. The same prognostic variables were also evaluated by multivariate analysis (Cox model) using a stepwise regression procedure. Statistical analysis system (SAS) statistical software (SAS Institute, Cary, NC) was used for all statistical analyses. $P$ less than .05 was considered statistically significant.

## RESULTS

### Clinical characteristics

Clinical data are listed in Table I. BPDC generally affects older patients; the age range of our patients was 5 to 90 years (median, 62.2 years). Twenty-one (80.8%) were older than 55 years, whereas only 3 (14.3%) were younger than 16 years. There were 19 male and 7 female patients for a ratio of 2.7 to 1.

Skin lesions of 23 patients (88.5%) were observed at the time of diagnosis. These skin lesions varied from 0.5 to 6 cm (usually at least 1 cm) in diameter, were purple in color, and had infiltrated the dermis. Lesions are usually described as macules, papules, nodules, or tumefactions, or as erythematous, highly pigmented, purpuric, necrotic, dark red, or purplish. No ulceration was detected in any of the patients (Fig 1). Only 3 patients (3, 17, and 20 in Table I) did not have cutaneous involvement at presentation. The staging procedure demonstrated that 19 of 24 (79.2%) peripheral blood, 13 of 19 (68.4%) bone marrow, 9 of 26 (34.6%) lymph nodes, and 9 of 26 (34.6%) samples of other tissues bore disease involving the central nervous system (3 patients), hepatosplenomegaly and pharynx (two patients each), orbit, parotid gland, lacrimal gland, and thyroid gland (one patient each). Asymptomatic bone-marrow infiltration was common; 15 of 21 patients (71.4%) showed thrombocytopenia, 10 of 17 (58.8%) anemia, 3 of 24 (12.5%) leukopenia, and 14 of 24 (58.3%) had leukocytosis.

### Morphologic, cytogenetic, and cytochemical features

Morphologic features were characterized by prominent, diffuse, and dermal infiltration of mononuclear cells, featuring epidermal sparing and formation of vague periadnexal and perivascular nodules that did not cause damage to the vessels.

The malignant cells usually consisted of monomorphic medium blasts with fine chromatin and agranular cytoplasm. In 10 cases, blast morphology was pleomorphic with cells varying from small to large with a regular-shaped round or oval nucleus (Fig 2, *A*). Mitotic figures were frequently observed (Fig 2, *B*). Morphologic analysis of bone-marrow aspirates most often showed hypercellular bone marrow with a high count of neoplastic cells in more than half of the patients. The cells usually contained a large amount of cytoplasm with a medium or low nucleus-cytoplasm ratio. The cytoplasm was average in quantity, slightly basophilic, and nongranular but displayed a heterogeneous structure, with a ring or "pearl necklace" of microvacuoles beneath the cytoplasmic membrane (Fig 3). Another frequent feature was the presence of pseudopodia-shaped cytoplasmic expansions. MPO and esterase reactions were negative.

### Immunophenotypic characterization of BPDC tumor cells

Immunophenotypic features of malignant cells are summarized in Tables II and III (Fig 2, *C* to *L*). Among the markers expressed by BPDC tumor cells, CD4 was found to be positive in all patients tested (26 of 26), CD56 in 96.2% (25 of 26), CD123 in 75% (18 of 24), and CD68 (KP-1) in 52.6% (10 of 19). The cells were negative for CD45RO, CD3, CD8, CD20, MPO, CD34, and CD1a.

Other markers frequently expressed by the tumor cells were CD33 (3 of 4; 75%), CD13 (2 of 4; 50%), CD14 (1 of 2; 50%), CD7 (7 of 14; 50%), CD2 (6 of 14; 42.9%), and CD11c (5 of 14; 35.7%). Terminal deoxynucleotidyl transferase was focally detected in two of 17 (11.8%) patients, T-cell intracellular antigen-1 in one of 13 (7.7%), and S-100 in one of 6 (16.7%).

In situ hybridization of EBV-encoded RNA (EBV-encoded nuclear small nonpolyadenylated RNA probes) for detection of EBV yielded negative results for all 13 patients tested.

Of the 22 BPDCs, 18 (81.8%) showed expression for TCL1 in skin (14/17; 82.4%), lymph nodes (4/5; 80%), and bone marrow (2/3; 66.7%). Positive cases showed strong uniform nuclear and cytoplasmic expression in 50% (10 of 20), including skin (8/14; 57.1%) and lymph nodes (2/4; 50%). Partial positivity was found in two cases. CLA was expressed in 5 of 23 (21.7%) patients in skin (3/18; 16.7%), lymph nodes (2/5; 40%), and bone marrow (1/3; 33.3%).

Immunohistochemistry was used to examine expression of chemokines and chemokine receptors. CXCL10 expression was seen in all 13 (100%) cases, CXCR4 in 19 of 21 (90.5%), CXCL9 in 16 of 22 (72.7%), CXCR3 in 15 of 22 (68.2%), CXCL12 in 12 of 20 (60%), CCR5 in 9 of 21 (42.9%), CCL2 in 7 of 21 (33.3%), CCR7 in 6 of 19 (31.6%), CCL5 in 4 of 16 (25%), and CCR4 in 0 of 11 (0%) BPDCs. The chemokine receptor CXCR4 and its corresponding ligand CXCL12 showed relatively high expression. The statistical significance of clinical progression could be determined in terms of the expression of chemokine stromal cell–derived factor-1 (CXCL12) of the CXCR4 ligand (Table IV and Fig 4). CXCL12 was positive in 8 of 15 skin biopsy specimens. Leukemic change after skin lesions occurred in 7 of 8 CXCL12-positive cases (87.5%) and in 3 of 6 CXCL12-negative cases (50%). Seven of 8 CXCL12-positive patients have died (87.5%), whereas 4 of 6 CXCL12-negative patients remain alive (66.7%). Statistically significant associations between overall survival and CXCL12 were identified. In the univariate Cox proportional hazards models, CXCL12-positive patients ran a significantly higher risk of death than CXCL12-negative patients (hazard ratio = 9.611; $P = .0151$). The findings for the multivariate Cox proportional hazards models also showed significant differences (hazard ratio = 14.901; $P = .0490$). Differences among the other chemokines and chemokine receptors were not statistically significant, whereas other clinical parameters (age and sex) showed no statistical differences between groups.

### Cytogenetics

Southern blot analysis showed TCR genes in the germline configuration in all but one case. Chromosomal analysis identified 3 cases as normal karyotypes and the remaining 4 cases as 47,XX, t(2;3)(p25;q21),del(6)(q?),del(17)(p?),+mar1 in all 3 cells of the first case, 45,X in 3 of 20 cells of the second case, 47,X,-Y,+8,+8 in 17 of 20 cells of the third case, and 46,XX,inv(9)(p11q13) in 17 of 20, 47,XX, +3,inv(9)(p11q13) in one of 20, 43,XX,add(9)(q34), inv(9)(p11q13),-10,-13,-15,add(18)(q21),-21,-22,+mar1, +mar2 in two of 20 cells, and 47,X,-Y,+8,+8 in 17 of 20 cells of the fourth case.

### Therapeutic response

First-line treatments varied widely (Table I) and included no treatment (two cases), localized radiation therapy (4 cases), prednisolone only (two cases), monochemotherapy (two cases), polychemotherapy (9 cases), and myeloablative therapy (4 cases: one cord blood stem cell transplantation, one autologous, and two allogeneic transplants). Complete remission after first-line therapy was achieved in 14 of 22 cases (63.6%), including one case of spontaneous regression regardless of the type of therapy administered. One patient (4.5%)

**Table I.** Clinical features, laboratory findings, and outcome of 26 patients with blastic plasmacytoid dendritic cell neoplasm

| Patient No. | Age (y) | Sex | Location: Skin | Location: LN | Location: BM | Initial skin appearance: No. | Initial skin appearance: state, form, size | Initial skin appearance: color | Initial skin appearance: location | Peripheral blood: WBC ($10^9$/L) | Peripheral blood: Blast (%) | First-line treatment | Response to first line | Second-line treatment | Type of therapy | Outcome | Survival (mo) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 46 | M | + | | | 1 | Nodule (2 cm) | Subcutaneous | Arm | | + | (AML-type) | PD | | AML-type | Dead | 1 |
| 2 | 57 | M | + | | + | Multiple | 0.5-3 cm | Erythematous | Whole body | 1.60 | 80.0 | DeVIC | CR | CHASE | Lymphoma-type | Dead | 6 |
| 3 | 57 | M | + | | + | Multiple | Nodules (2 cm) | Subcutaneous | Trunk | 109.70 | 96.0 | Allogeneic transplant | PD | | Transplant | Dead | 13 |
| 4 | 59 | F | + | + | | 1 | Tumor (5 cm) | | Neck | 5.38 | 0.0 | RT | CR | PSL, MACOP-B | Lymphoma-type | Dead | 6 |
| 5 | 71 | M | + | + | + | 2 | 2 cm, 6 cm | Erythematous | Face and back | 2.50 | 0.0 | CHOP | PD | ABEP, LDVP16 | Lymphoma-type | Dead | 5 |
| 6 | 72 | M | + | + | | Multiple | Plaques | Erythematous | | 4.70 | 60.0 | PSL, RT | PD | VP | Lymphoma-type | Dead | 3 |
| 7 | 73 | M | + | | + | Multiple | | Subcutaneous | Whole body | 66.50 | 19.0 | LDAC | PD | MIT | AML-type | Dead | 6 |
| 8 | 74 | M | + | | + | Multiple | Nodules (1 cm) | Erythematous purple | Except scalp | 30.50 | 9.0 | PSL | PR | CA, high-dose AraC | AML-type | Dead | 5 |
| 9 | 79 | F | + | + | + | Multiple | Macules (1-2 cm) | Erythematous purple | Whole body | 17.90 | 42.0 | THP-COP | CR | DeVIC, PSL | Lymphoma-type | Dead | 18 |
| 10 | 84 | F | + | | | Multiple | Plaques (3 cm) | Erythematous purple | Scalp | 22.53 | 10.0 | THP-COP | PD | | Lymphoma-type | Dead | 4 |
| 11 | 85 | M | + | + | | Multiple | | Erythematous purple | Leg | 20.80 | 33.0 | None | PD | | | Dead | 7 |
| 12 | 85 | M | + | + | + | Multiple | | Purple | Scalp, trunk, and arms | 42.80 | 80.0 | THP-COP | CR | ABEP, ESHAP, LDVP16 | Lymphoma-type | Dead | 25 |
| 13 | 88 | M | + | | + | Multiple | | Erythematous purple | Face, trunk, and arms | 10.10 | 22.0 | PSL | CR | VP16 | Lymphoma-type | Dead | 12 |
| 14 | 72 | F | + | | | Multiple | 1 cm | | Trunk and extremities | 4.60 | 27.0 | Unknown | Unknown | | | Dead | Unknown |
| 15 | 75 | M | + | | | Multiple | | Erythematous purple | Except scalp | 3.69 | 3.0 | THP-COP | CR | | Lymphoma-type | Dead[‡] | 12 |
| 16 | 14 | M | + | | | Multiple | 2-4 cm | Subcutaneous | Extremities | 4.50 | 0.0 | Unknown | Unknown | | | Unknown | Unknown |
| 17 | 61 | M | | + | | | | | | 12.00 | 23.0 | Unknown | Unknown | | | Unknown | Unknown |
| 18 | 73 | M | + | | | Multiple | Nodules | Subcutaneous | Whole body | 10.00 | 0.0 | Unknown | Unknown | | | Unknown | Unknown |
| 19 | 5 | F | + | | | 1 | | Subcutaneous | Arm | | Unknown | (ALL-type) | CR | | ALL-type | Alive | 12 |
| 20 | 15 | F | | + | + | | | | | 10.63 | 34.5 | None | CR* | AraC, VP16, MIT, TIT, JPLSG AML05 | AML-type | Alive | 18 |
| 21 | 35 | M | + | | + | Multiple | Nodules (1-2 cm) | | Trunk | 61.90 | 97.5 | Autologous transplant | CR | | Transplant | Alive | 31 |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | 57 | M | + | + | + | Multiple | 3 cm | Erythematous | Trunk and extremities | 2.96 | + | | ALL MRD2002 protocol, CBSCT | CR | | ALL-type transplant | Alive | 21 |
| 23 | 58 | M | + | | | Multiple | Plaques (2-3 cm) | Erythematous | Trunk and extremities | 5.10 | | 0.0 | Allogeneic transplant | CR | | Transplant | Alive | 42 |
| 24 | 60 | M | + | | | 1 | Plaques (6 cm) | | Back | 5.14 | | 0.0 | RT | CR | | | Alive | 7 |
| 25 | 73 | M | + | | + | Multiple | Plaques | Erythematous | Trunk and extremities | 82.78 | | 92.5 | CHOP | CR | | Lymphoma-type | Alive | 11 |
| 26 | 90 | F | + | | + | Multiple | | | Trunk and arms | 11.60 | | 34.0 | VP16 and RT | CR | CHOP | Lymphoma-type | Alive | 14 |

Unspecified treatment protocols were described as parentheses.

*ABEP*, Aclarubicin, enocitabine, etoposide, and prednisolone; *ALL*, acute lymphoblastic leukemia; *AML*, acute myeloid leukemia; *AraC*, cytosine arabinoside; *BM*, bone marrow; *CA*, cytosine arabinoside and aclacinomycin; *CBSCT*, cord blood stem cell transplantation; *CHASE*, cyclophosphamide, high-dose cytosine arabinoside, etoposide, and dexamethasone; *CHOP*, cyclophosphamide, Adriamycin, vincristine, and prednisolone; *CR*, complete remission; *DeVIC*, dexamethasone, etoposide, ifosfamide, carboplatin; *ESHAP*, etoposide, methylprednisolone, cisplatin, and cytosine arabinoside; *F*, female; *JPLSG*, Japanese Pediatric Leukemia/Lymphoma Study Group; *JPLSG AML-05*, A Multi-Center Phase II Study in Children with Newly Diagnosed Acute Myeloid Leukemia of JPLSG protocol; *LDAC*, low-dose cytosine arabinoside; *LDVP16*, low-dose etoposide; *LN*, lymph nodes; *M*, male; *MACOP-B*, methotrexate, leucovorin, doxorubicin, cyclophosphamide, vincristine, prednisolone, and bleomycin; *MIT*, mitoxantrone; *MRD*, minimal residual disease; *PD*, progressive disease; *PR*, partial response; *PSL*, prednisolone; *RT*, radiation therapy; *THP-COP*, pirarubicin, cyclophosphamide, vincristine, and prednisolone; *TIT*, triple intrathecal therapy; *VP*, vincristine and predonisolone; *VP16*, etoposide; *WBC*, white blood cell count.

*Spontaneous regression.

†Dead of lung cancer.

achieved partial response, and no response was observed in 7 patients (31.8%). To date, 8 of 14 (57.1%) patients who achieved complete remission have experienced a relapse. Of the 22 patients treated, two (9.1%) received ALL-type therapy, 4 (18.2%) AML-type therapy, and 11 (50%) lymphoma-type therapy. Three of the 4 transplanted patients were still alive at the end of this study. Median follow-up was 12.7 months (range, 1-42 months).

## DISCUSSION

Malignant lymphoid neoplasms with blastic morphology $CD4^{+}CD56^{+}$ but a non-T non-B phenotype share a heterogeneous spectrum of clinically aggressive entities.

BPDCs were formerly referred to as blastic NK-cell lymphomas on the basis of their CD56 expression.[23-25] In recent years, however, accumulating evidence has supported a link between pDCs and the origin of BPDC[1,6,9,26-28] and this is included under the term "blastic plasmacytoid dendritic cell neoplasm" in the chapter on AML of the 4th edition (2008) of the "WHO classification of tumors of hematopoietic and lymphoid tissues."[12]

Our report concerns the findings for 26 Japanese patients with BPDCs. The majority of the patients were older adults[2,6,7,29,30] but the disease may occur in younger adults or in children as also previously reported.[2,7,9,25,31-34] Most patients presented with cutaneous lesions at initial examination,[2,6] which spread within a few months to involve general areas of the cutis, and blood, bone marrow, and lymph nodes.[8] Cytopenia, especially thrombocytopenia, was common[2,9,29,30,35] and leukocytosis was also noted in some patients. Although the health status of most patients is good without systemic symptoms at diagnosis, progression of the disease typically leads to the patient's death within a few years after initial examination.[1-3,6,9,26-28] At present there is no consensus on optimal treatment for BPDC and therapeutic approaches for BPDC vary widely from radiation to myeloablative therapy. Despite initial good response to various therapies, outcome is generally poor.[6,7,30,35] However, allogeneic stem cell transplantation should be considered the first-line treatment whenever feasible.[2,4-6,9,27,34,36,37] In 2009, Tsagarakis et al[30] reported that the basic conclusion derived from their clinical findings was the superiority of ALL-type regimens for BPDC treatment. Because allogeneic stem cell therapy is limited to younger patients and most patients with BPDC are in their seventh decade of life, the vast majority of patients are unfortunately not eligible for this promising therapeutic option. Three of the 4 transplanted patients in our study were alive at the time of writing. For

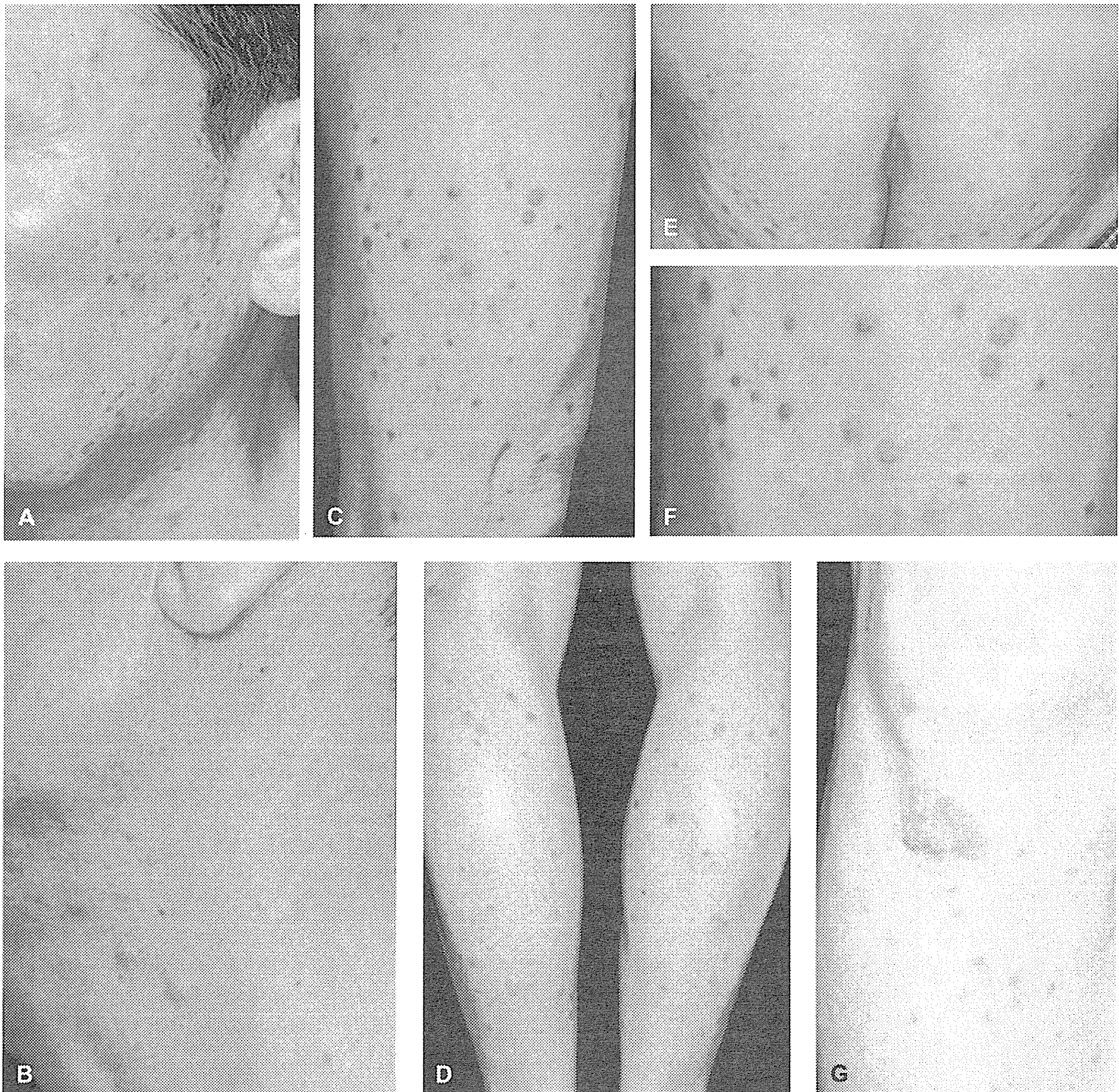


**Fig 1.** Clinical features, patient 8. Multiple dermal papules and nodules appeared on temple (**A**) and neck (**B**), as did violaceous and violaceous nodules on right (**C**) and flexor (**D**) legs, and buttock (**E**). **F**, High-power view of **C**. **G**, Erythematous plaque below the right knee.

patients not eligible for allogeneic stem cell transplantation, however, the optimal therapy remains unknown.

BPDC can usually be identified by the following phenotype: CD45 low, CD4$^+$, CD56$^+$, CD116 low, CD123 high, HLA-DR$^+$, CD45RA$^+$, CD45RO$^-$, blood dendritic cell antigen-2$^+$ (CD303$^+$), blood dendritic cell antigen-4$^+$ (CD304$^+$), immunoglobulin-like transcript-3$^+$.[1,2,9,38-40] For diagnosis, the characteristic phenotype of malignant pDCs shows CD4$^-$ CD56$^-$ and CD123$^+$ cell surface antigens. However, at least 11 cases of BPDC reported in the literature atypically lacked CD4 expression,[4,33,41,42] so that CD56 appears to be a very useful, although not critical, marker for diagnosis. However, a few CD56$^-$ cases have also been reported.[27,35,38,43-45] In our study, all cases expressed CD4 but one case lacked CD56.

TCL1 is an Akt kinase regulator and lymphoid protooncogene, with nonneoplastic expression restricted to pDC and B cells.[46-49] In reactive lymph nodes, the only other TCL1$^+$ cells noted apart from B

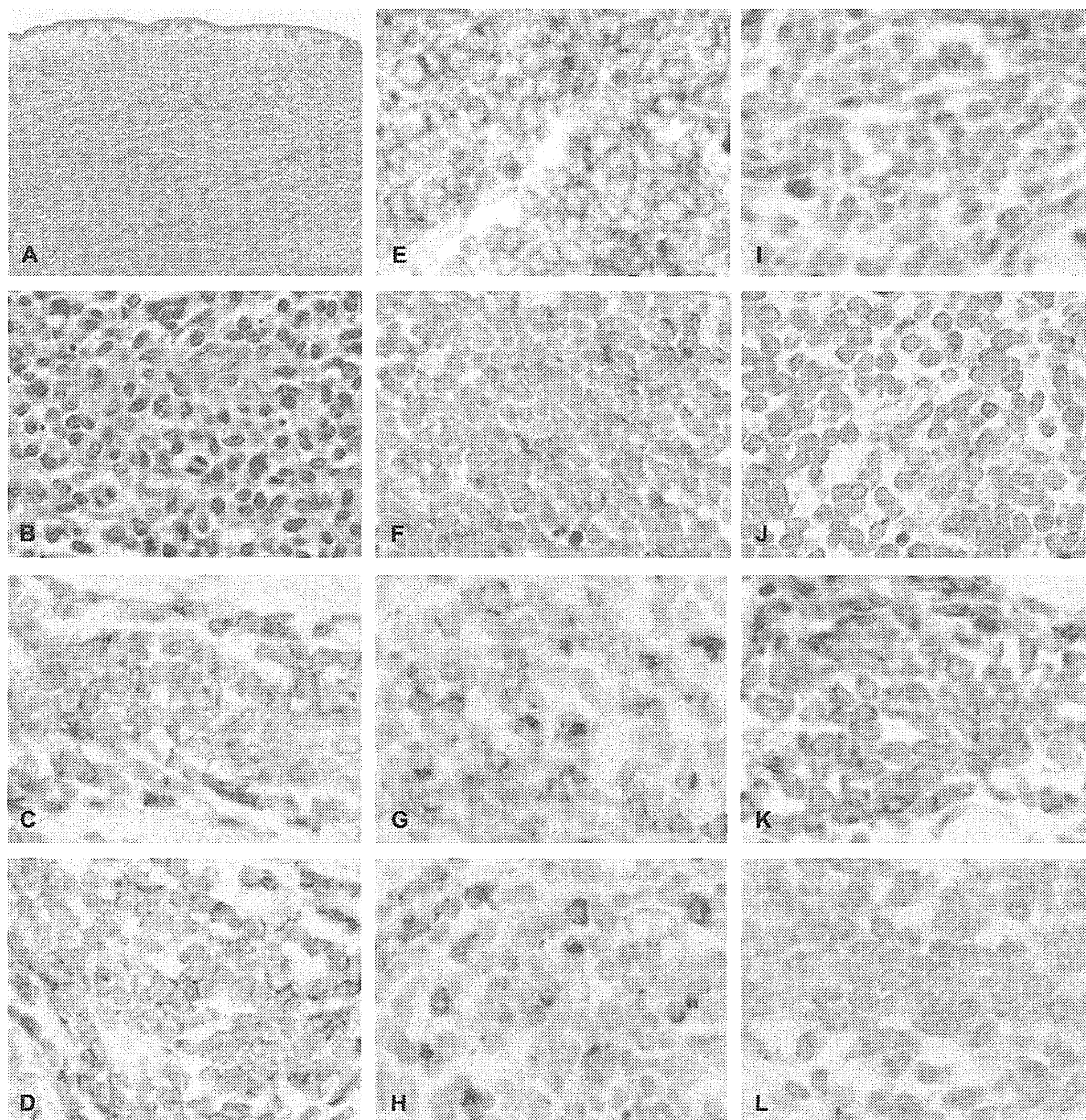


**Fig 2.** Pathological features. **A**, Skin biopsy specimen shows diffuse, dense, atypical dermal lymphoid infiltrate sparing epidermis. **B**, Neoplastic cells in skin biopsy specimen are small to intermediate with round to irregular nuclear contours and finely dispersed chromatin. Mitotic features are frequently seen. Neoplastic cells in skin show expression for CD4 (**C**), CD56 (**D**), CD123 (**E**), cutaneous lymphocyte-associated antigen (**F**), CXCR3 (**G**), CXCL9 (**H**), CXCL10 (**I**), CXCR4 (**J**), CXCL12 (**K**), and CCR5 (**L**). Photographs are of patient 8 (**A, B, F** to **I, K,** and **L**), patient 6 (**C** to **E**), and patient 13 (**J**). (**A** and **B**, Hematoxylin-eosin stain; original magnifications: **A**, ×100; **B**, ×400.)

cells were clusters of pDCs, which also expressed CD4 (dim), CD45RA (dim), CD68, and CD123.[11] Because the possible ontogeny of BPDCs as DC precursors and their immunophenotype seems to have been established, CD123 expression suggests that this disease also derives from plasmacytoid monocytes.[50,51]

BPDCs and their myelomonocytic transformations highly express TCL1, a feature shared with pDCs but not with myelomonocytic leukemia,[10,11]

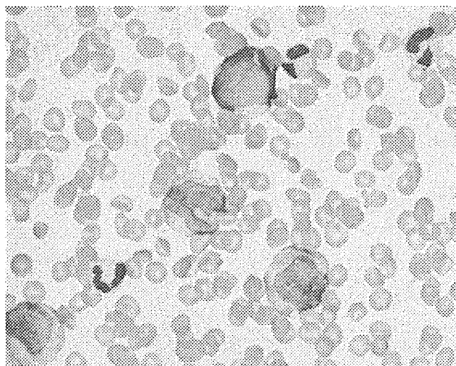

**Fig 3.** Cytologic features of blastic plasmacytoid dendritic cell. Neoplastic cells in bone-marrow aspirate (patient 8) have intermediate-sized nuclei with fine chromatin and moderate amount of pale, agranular cytoplasm. These cells often display cytoplasmic microvacuoles arranged as pearl necklace along cytoplasmic outline. (Wright-Giemsa stain; original magnification: ×1000.)

true NK-cell tumors, or mature T-cell malignancies except for T-cell prolymphocytic leukemia.[11] Herling et al[11] demonstrated that BPDC blasts and myelomonocytic blasts arose from a common leukemic clone because they both expressed the TCL1 proto-oncogene, which is a marker never encountered in de novo myeloid leukemias. In addition, it was reported that BPDCs express higher levels of CD123 than do other types of acute leukemias as determined by cytometry and assessed by fluorescence intensity ratio.[29,52] Immunostaining has demonstrated strong staining for CD123 in more than 90% of BPDCs,[3,6,9,28] whereas weak expression of CD123 has been found in myelomonocytic leukemias.[7] Thus, CD123 and TCL1 represent useful markers for identifying BPDCs and distinguishing them from other CD56$^+$ cutaneous tumors, including extranodal NK-cell lymphomas of nasal-type and myelomonocytic leukemias. For routine phenotypic determination of pDC-specific markers, CD123 is thus very useful.

As already mentioned,[10,11] we noted in 18 (81.8%) of 22 cases (14/17 skin, 4/5 lymph node, 2/3 bone marrow) variable immunohistochemical positivity for TCL1, which showed strong expression in 10 cases (skin: 8, lymph node: 2). CD123 was also strongly positive in 75% of cases examined (18 of 24).

It has been suggested that any potential homing tendency of CLA (HECA-452 antibody) to skin is not restricted to lymphocytes but also may occur in other cell types such as BPDCs. Although CLA alone cannot discriminate between BPDC and cutaneous AML, it may perform an important role in lymphocyte homing to the skin.[53,54] In our series, we found that only 5 of 23 cases (21.7%) of BPDC were positive for CLA. In the

**Table II.** Immunophenotypic findings of 26 patients with blastic plasmacytoid dendritic cell by flow cytometry or/and immunohistochemical examination

| Patient No. | Site | CD4 | CD56 | CD123 | CD68 | TCL1 | EBV |
|---|---|---|---|---|---|---|---|
| 1 | Skin | + | + | − | NA | − | NA |
| 2 | Skin | + | + | + | + | + | − |
| 3 | Skin | + | − | + | NA | − | NA |
| | BM | + | − | + | NA | − | NA |
| 4 | Skin | + | + | NA | − | ± | − |
| 5 | LN | + | + | + | − | + | − |
| 6 | Skin | + | + | + | − | +S | NA |
| 7 | Skin | + | + | − | + | + | NA |
| 8 | Skin | + | + | + | + | + | − |
| | BM | + | + | NA | NA | ± | NA |
| 9 | LN | + | + | + | NA | +S | NA |
| 10 | Skin | + | + | + | + | +S | NA |
| 11 | Skin | + | + | + | NA | +S | NA |
| 12 | LN | + | + | + | − | +S | − |
| 13 | Skin | + | + | + | + | +S | − |
| 14 | Skin | + | + | − | − | ± | NA |
| 15 | Skin | + | + | NA | NA | NA | NA |
| 16 | Skin | + | + | + | − | NA | − |
| 17 | LN | + | + | + | + | + | − |
| 18 | Skin | + | + | − | + | NA | NA |
| 19 | Skin | + | + | + | + | +S | NA |
| 20 | LN | + | + | − | + | − | NA |
| 21 | Skin | + | + | + | NA | − | NA |
| 22 | Skin | + | + | + | − | NA | − |
| 23 | Skin | + | + | + | − | +S | − |
| 24 | Skin | + | + | + | − | +S | − |
| 25 | Skin | + | + | − | + | + | − |
| | BM | + | + | − | + | + | NA |
| 26 | Skin | + | + | + | NA | +S | − |
| Positive % | | 100 | 96.2 | 75 | 52.6 | 81.8 | 0 |

*BM*, Bone marrow; *EBV*, Epstein-Barr virus; *LN*, lymph nodes; *NA*, no data available; *+S*, strong; *TCL1*, T-cell leukemia 1.

literature, at least 50 BPDC cases (about 10% to 20% of all BPDC cases) demonstrated a transformation to myelomonocytic chronic and AML,[2,6,7,11,27,30,37,55-61] although to our knowledge no transformation of BPDCs to ALL has been reported.

Normal human peripheral blood lymphoplasmacytoid DCs have recently been shown to express several lymphoid-associated markers, such as CD2, CD4, CD7, and CD22,[62] along with the myeloid-associated marker CD33 in the absence of MPO. Furthermore, previous studies revealed that pDCs cultured in granulocyte/macrophage colony-stimulating factor or interleukin-3 induced CD13, CD33, and CD11c myeloid antigens[1,63] and that BPDCs and normal circulating pDCs[64-67] express the myeloid marker CD33.[1,39,63] In our study, conventional myeloid markers, except for MPO and c-kit, frequently expressed by tumor cells were CD33

**Table III.** Immunohistochemical examination for cutaneous lymphocyte-associated antigen, chemokines, and chemokine receptors

| Patient No. | Site | CLA | CXCR3 | CXCL9 | CXCL10 | CXCR4 | CXCL12 |
|---|---|---|---|---|---|---|---|
| 1 | Skin | + | − | + | NA | + | + |
| 2 | Skin | − | − | + | + | − | + |
| 3 | Skin | ± | − | − | + | + | − |
| | BM | + | − | − | + | + | + |
| 4 | Skin | − | ± | + | + | + | − |
| 5 | LN | − | − | ± | + | + | − |
| 6 | Skin | − | ± | ± | NA | + | + |
| 7 | Skin | − | ± | ± | + | NA | NA |
| 8 | Skin | − | ± | ± | + | − | + |
| | BM | − | − | + | + | + | − |
| 9 | LN | − | ± | − | NA | + | + |
| 10 | Skin | − | − | − | + | − | + |
| 11 | Skin | − | + | ± | NA | + | + |
| 12 | LN | ± | ± | ± | NA | + | − |
| 13 | Skin | − | + | − | + | + | NA |
| 14 | Skin | − | − | − | + | + | + |
| 15 | Skin | − | + | + | NA | + | − |
| 16 | Skin | NA | NA | NA | NA | NA | NA |
| 17 | LN | + | + | + | + | + | ± |
| 18 | Skin | NA | NA | NA | NA | NA | NA |
| 19 | Skin | NA | NA | NA | NA | NA | NA |
| 20 | LN | − | ± | ± | NA | + | − |
| 21 | Skin | − | ± | ± | NA | + | − |
| 22 | Skin | − | NA | NA | NA | NA | NA |
| 23 | Skin | ± | − | − | + | + | − |
| 24 | Skin | − | ± | ± | + | + | + |
| 25 | Skin | − | ± | ± | NA | + | − |
| | BM | − | ± | − | + | + | + |
| 26 | Skin | − | + | ± | NA | + | − |
| positive % | | 21.7 | 68.2 | 72.7 | 100.0 | 90.5 | 60.0 |

*BM*, Bone marrow; *CLA*, cutaneous lymphocyte-associated antigen; *LN*, lymph nodes; *NA*, no data available; +*S*, strong.

**Table IV.** Statistical analysis for overall survival

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | *P* value | HR | *P* value | HR |
| Age | | | | |
| −15 | | | | |
| 16-64 | 0.3397 | - | 0.9552 | - |
| 65- | | | | |
| Sex | 0.6103 | - | 0.2247 | - |
| Male | | | | |
| Female | | | | |
| CXCR4 | 0.0725 | - | 0.7869 | - |
| Negative | | | | |
| Positive | | | | |
| CXCL12 | 0.0151 | 9.611 | 0.0490 | 14.901 |
| Negative | | | | |
| Positive | | | | |

*HR*, Hazard ratio.

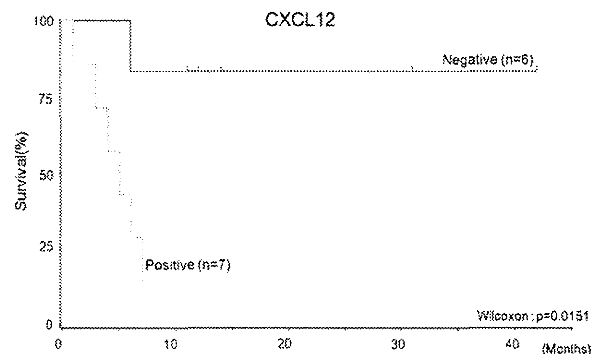


**Fig 4.** Kaplan–Meier curves for overall survival in relation to CXCL12. CXCL12-positive patients showed significantly higher risk of death than CXCL12-negative patients (hazard ratio = 9.611; *P* = .0151).

(3 of 4; 75%), CD13 (2 of 4; 50%), CD14 (1 of 2; 50%), and CD11c (5 of 14; 35.7%). Expression of CD68, CD7, and CD2 on normal pDCs has also been reported.[50,68,69] In addition, CD68 (PGM-1 and KP-1) in our study was positive in 52.6% (10 of 19), CD7 in 50% (7 of 14), and CD2 in 42.9% (6 of 14) of the cases. These results also support a common origin of pDC and the myeloid lineage.

Chemokine receptors mediate the migration, activation, and proliferation of lymphocytes through the binding of ligands, and their expression is differentially regulated in lymphocyte subsets. CXCR3 is known to be expressed on several types of cells in the hematopoietic lineage, including not only memory/activated lymphocytes and NK cells but also pDCs. The ligands of CXCR3, CXCL9, and CXCL10 induce chemotaxis and adhesion of cells, and are not only lymphocyte-dedicated chemokines. CXCL10 has been shown to promote the induction of T-helper 1 cytokines (eg, interferon-γ), which are necessary for an efficient antitumor response.[70] In addition, CXCL10 was previously recognized as an inhibitor of tumor angiogenesis.[71,72] CXCL9 was also found to inhibit neovascularization in vivo and to have antitumor effects.[73-75] In our study, BPDCs showed relatively high positivity for CXCR3 (68.2%) and its ligands CXCL9 (72.7%) and CXCL10 (100%), which may be associated with pDC origin, whereas it is also possible that the high positivity for CXCR3, CXCL9, and CXCL10 reflects immune response to BPDC malignancy.

CXCL12 has been found to be chemotactic for human T and B lymphocytes, monocytes, $CD34^+$ hematopoietic progenitor cells, dendritic cells, and megakaryocytes.[76-79]

CXCR4 activity is regulated by its interaction with the ligand CXCL12, and this CXCR4–CXCL12 axis is essential for leukocyte trafficking[76] and can mediate both proliferative[80] and apoptotic stimuli[81,82] in

normal hematopoietic cells. Normal pDCs migrate only in response to CXCL12[83,84] and not in response to any of the ligands of other expression receptors such as CXCR3. The migration of pDCs to CXCL12 is substantial, whereas CXCL12 also seems to play a relevant role in several types of lymphomas and leukemias.

In fact, CXCL12 enhances migration of follicular lymphoma cells,[13] and the CXCR4-CXCL12 circuitry appears to be crucial for migration of chronic lymphocytic leukemia[14,15] and ALL cells.[16]

Furthermore, the CXCR4—CXCL12 axis is involved in the metastasis of breast,[85] lung,[86] ovarian,[87] pancreatic,[88] neuroblastoma,[89] renal,[90] thyroid,[91] rhabdomyosarcoma,[92] prostate,[93] and colorectal[94] cancers along with lymphomas and leukemias.[13,15,95,96]

Recent findings also suggest that CXCL12 may be involved in breast cancer cell pseudopodia formation and in invasive breast cancer metastasis.[85] Previous studies of lymphoma cells have also demonstrated that pseudopodia formation is crucial for tissue invasion and metastasis formation.[95,97] BPDCs also frequently present cytoplasmic expansions resembling pseudopodia.[6,7,29,30] CXCL12 may therefore be associated with the migration of BPDCs through pseudopodia formation and leukemic changes.

In our study, leukemic change after skin lesions occurred more frequently in 7 of 8 CXCL12-positive cases (87.5%) than in 3 of 6 CXCL12-negative cases (50%). Seven of 8 CXCL12-positive patients (87.5%) and two of 6 CXCL12-negative patients (33.3%) died, whereas one of 8 CXCL12-positive patients (12.5%) and 4 of 6 CXCL12-negative patients (66.7%) remained alive. Furthermore, multivariate analysis (CXCL12, CXCR4, age, and sex) demonstrated that CXCL12-positive patients showed a 14.901-fold higher risk of death than did CXCL12-negative patients.

In conclusion, our findings for high CXCL12 expression in BPDC indicate that it is associated with systemic dissemination and poor prognosis, thus making the CXCR4-CXCL12 circuitry an attractive target for BPDC therapies. Further studies are thus clearly warranted to determine the efficacy of this strategy.

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Journal of Pathology
J Pathol 2012; **228:** 1–7
Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.4062

INVITED COMMENTARY

# Pathogenesis of epidermolysis bullosa acquisita, an autoimmune subepidermal bullous disease[#]

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## Abstract

Autoimmune bullous diseases (ABDs) are organ-specific autoimmune diseases, in which blisters on the skin and mucous membranes develop through binding of pathogenic autoantibodies to target antigens. There are two major ABD groups: the pemphigus group, showing autoantibodies to desmosomal components; and the subepidermal ABD group, showing autoantibodies to hemidesmosomal components in the epidermal basement membrane zone. Recent immunological, biochemical and molecular biological studies revealed many new autoantigens, including desmocollins, various plakin family proteins and integrins. A revised ABD classification includes new disease entities such as paraneoplastic pemphigus, IgA pemphigus and anti-laminin γ1 pemphigoid. In addition to systemic corticosteroids and various immunosuppressive agents, various adjuvant therapies for ABDs have developed. Among them, intravenous immunoglobulin (IVIG) is a promising therapy, although the therapeutic mechanisms are still unknown. Various disease models for ABDs have developed, particularly for pemphigus vulgaris, bullous pemphigoid and epidermolysis bullosa acquisita (EBA), and these have provided insights into the pathogenesis of various ADBs that suggest possible new treatment strategies. However, the fundamental mechanisms in disruption of immune-tolerance are still unknown. EBA shows autoimmunity to type VII collagen, the major component of anchoring fibrils, and EBA pathogenesis has been studied in various disease models. Previous studies suggested that, following binding of autoantibodies to type VII collagen, activation of complement, cytokine release, neutrophil migration, Fcγ receptors (FcgRs) and metalloproteinases play important roles in induction of subepidermal blisters. In this issue of the *Journal of Pathology*, Kasperkiewicz and colleagues reveal important roles of activating FcgRIV and inhibitory FcgRIIB in EBA pathogenesis that were recognized by conducting elegant studies using both genetic analysis and functional animal model methods. The expression equilibrium of the activating and inhibitory FcgRs can be modulated towards the inhibitory FcgRIIB by IVIG therapy, resulting in beneficial clinical effects of IVIG in EBA and other autoimmune skin-blistering diseases.
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**Keywords:** autoantibody; autoimmune bullous disease; disease model; epidermolysis bullosa acquisita; FcgR; intravenous immunoglobulin; leukocyte; pathogenesis; therapy

[#] *Invited commentary for Kasperkiewicz M, Nimmerjahn F, Wende S, et al. Genetic identification and functional validation of FcγRIV as key molecule in autoantibody-induced tissue injury. J Pathol 2012; DOI: 10.1002/path.4027.*

Received 13 March 2011; Revised 8 May 2012; Accepted 28 May 2012

*No conflicts of interest were declared.*

## Introduction

Strong cell adhesion both between keratinocytes and at the epidermal basement membrane zone (BMZ) is crucial to maintain the normal integrity of the epidermis that forms the outermost part of the skin [1–3] (Figure 1a–c). There are two major cell adhesion apparatuses, ie desmosomes for adhesion between keratinocytes and hemidesmosomes for adhesion between the epidermis and the underlying dermis (Figure 1a–c).

Desmosomes have three groups of constituent proteins, ie the desmosomal cadherin family consisted of desmogleins 1–4 (Dsg1–4) and desmocollins 1–3 (Dsc1–3), the plakin family consisting of epiplakin, desmoplakin I/II, envoplakin and periplakin, and the armadillo family consisting of plakoglobin and plakophilins. At the epidermal BMZ, there are many molecules at hemidesmosomes, lamina lucida, lamina densa and anchoring fibrils in the uppermost dermis. In hemidesmosomes, plectin and BP230 are present at the intracellular attachment plaques, and BP180 and α6β4 integrin serve as linkers between keratinocytes and various extracellular matrices at epidermal BMZ. Major extracellular matrix proteins within the BMZ are laminin-332, laminin trimers containing laminin γ1, type IV collagen and type VII collagen (collagen VII encoded by *COL7A1*), which is the major component of anchoring fibrils.

These molecules are important in maintaining normal skin structure, because genetic diseases involving these proteins, mainly various types of inherited

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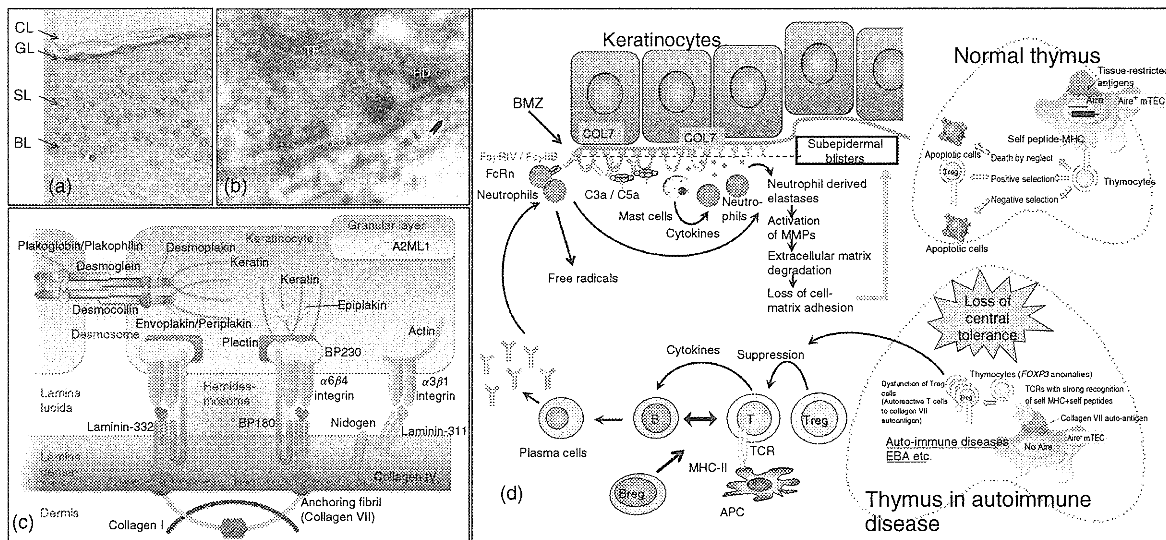


Figure 1. (a) Histopathological view of the normal human skin (H&E stain). The layers are shown in the left: CL, cornified layer/stratum corneum; GL, granular layer/stratum granulosum; SL, spinous layer/stratum spinosum; BL, basal layer/stratum basale. (b) Electron-microscopic view of lower part of a basal cell: TF, tonofilaments/keratin intermediate filaments; HD, hemidesmosomes; LL, lamina lucida; LD, lamina densa; AF, anchoring fibrils. (c) Schematic figure of basal cells presenting structures and constituent components of both desmosomes and hemidesmosomes. Most of these molecules are autoantigens for various ABDs. (d) Schematic figures for pathological mechanisms in ABDs, particularly in EBA. General consensus for intra-thymus events (right area) and extra-thymus events (lower-left area) for possible induction processes for autoimmunity (loss of immune tolerance) are depicted. Possible mechanisms for blister formation in EBA are also shown in the upper-left area: COL7/collagen VII, type VII collagen; mTECs, medullar thymic epithelial cells; TCR, T cell receptor; Treg, regulatory T cells; Breg, regulatory B cells; MHC-II, major histocompatibility complex class II; APCs, antigen-presenting cells.

epidermolysis bullosa, result in severe blister formation [3]. In addition, most of these molecules were also shown to be autoantigens in various autoimmune bullous diseases (ABDs).

ABDs are potentially life-threatening skin diseases, clinically characterized by blisters and erosions on the skin and mucous membranes. Patients with ABDs develop autoantibodies reactive with epidermal keratinocyte cell surfaces or the epidermal BMZ, which in turn induce separation between epidermal keratinocytes or at the BMZ [4]. Based on clinical, histopathological and immunological criteria, ABDs are classified into two major groups; ie the pemphigus group associated with autoantibodies to desmosomal components, and subepidermal ABDs with autoantibodies to hemidesmosomal components in the BMZ (Figures 1a–c, 2).

Patients suffering from ABDs are treated mostly with systemic corticosteroids and various immunosuppressive drugs [5]. These treatments are effective in most cases, but often show various serious adverse effects, including systemic infections, gastrointestinal disorders, osteoporosis, psychiatric disorders, hypertension, hyperlipidaemia, diabetes mellitus, moon face and obesity, which contribute significantly to the increased mortality of these patients [6,7]. Therefore, there is a great, and so far unmet, medical need for safer and more effective treatment modalities for ABDs. Recently, several new promising therapeutic agents and modalities have been reported. Intravenous immunoglobulin (IVIG) has been used as an effective therapy for various autoimmune-inflammatory diseases, including autoimmune thrombocytopenia [8], Guillain–Barré syndrome [9], multiple sclerosis [10], myasthenia gravis [11] and Kawasaki disease [12]. IVIG is also effective in autoimmune skin diseases, including dermatomyositis, systemic lupus erythematosus (SLE) and ABDs [13–17]. IVIG is successfully used in severe and recalcitrant patients with various ABDs, although the therapeutic mechanisms are not fully understood.

In this commentary, we first summarize the recent progress in various aspects of ABDs, including new classification with associated autoantigens, pathogenesis, disease models and therapeutic modalities, particularly pathological features and disease models in EBA, a subepidermal type of ABD reactive with collagen type VII (COL7). Subsequently, we focus on therapeutic mechanisms of IVIG and roles of FcgRs on the blister formation in EBA.

## Classification of ABDs

In the current classification for ABDs, there are a number of different disease entities within both the pemphigus and subepidermal ABD groups (Figure 2). Among them, pemphigus vulgaris (PV), pemphigus foliaceus, bullous pemphigoid (BP) and dermatitis herpetiformis are the classical ABDs. EBA is also well characterized, both clinically and pathologically.

The autoantigens for the major ABDs were identified decades ago, including Dsgs in classical pemphigus [18], BP230 and BP180 in BP [19] and COL7

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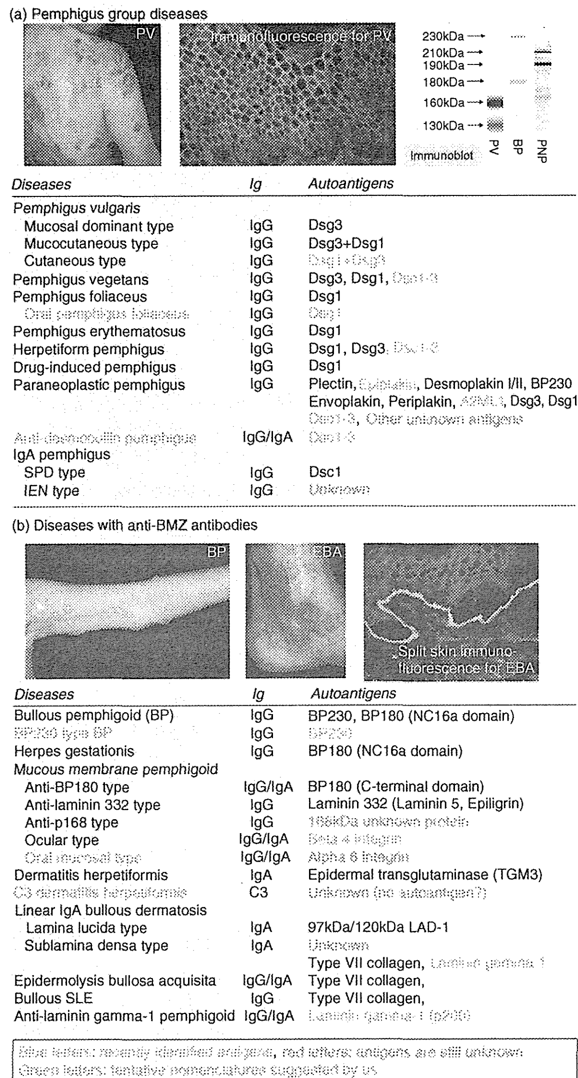


| *Diseases* | *Ig* | *Autoantigens* |
|---|---|---|
| *Pemphigus vulgaris* | | |
| Mucosal dominant type | IgG | Dsg3 |
| Mucocutaneous type | IgG | Dsg3+Dsg1 |
| Cutaneous type | IgG | Dsg1+Dsg3 |
| Pemphigus vegetans | IgG | Dsg3, Dsg1, Dsc1-3 |
| Pemphigus foliaceus | IgG | Dsg1 |
| Oral pemphigus foliaceus | IgG | Dsg1 |
| Pemphigus erythematosus | IgG | Dsg1 |
| Herpetiform pemphigus | IgG | Dsg1, Dsg3, Dsc1-3 |
| Drug-induced pemphigus | IgG | Dsg1 |
| Paraneoplastic pemphigus | IgG | Plectin, Epiplakin, Desmoplakin I/II, BP230 Envoplakin, Periplakin, A2ML1, Dsg3, Dsg1 Dsc1-3, Other unknown antigens |
| Anti-desmocollin pemphigus | IgG/IgA | Dsc1-3 |
| IgA pemphigus | | |
| SPD type | IgG | Dsc1 |
| IEN type | IgG | Unknown |

| *Diseases* | *Ig* | *Autoantigens* |
|---|---|---|
| Bullous pemphigoid (BP) | IgG | BP230, BP180 (NC16a domain) |
| BP230 type BP | IgG | BP230 |
| Herpes gestationis | IgG | BP180 (NC16a domain) |
| *Mucous membrane pemphigoid* | | |
| Anti-BP180 type | IgG/IgA | BP180 (C-terminal domain) |
| Anti-laminin 332 type | IgG | Laminin 332 (Laminin 5, Epiligrin) |
| Anti-p168 type | IgG | 168kDa unknown protein |
| Ocular type | IgG/IgA | Beta 4 integrin |
| Oral mucosal type | IgG/IgA | Alpha 6 integrin |
| Dermatitis herpetiformis | IgA | Epidermal transglutaminase (TGM3) |
| C3 dermatitis herpetiformis | C3 | Unknown (no autoantigen?) |
| Linear IgA bullous dermatosis | | |
| Lamina lucida type | IgA | 97kDa/120kDa LAD-1 |
| Sublamina densa type | IgA | Unknown |
| | | Type VII collagen, Laminin gamma 1 |
| Epidermolysis bullosa acquisita | IgG/IgA | Type VII collagen, |
| Bullous SLE | IgG | Type VII collagen, |
| Anti-laminin gamma-1 pemphigoid | IgG/IgA | Laminin gamma-1 (p200) |

Blue letters: recently identified antigens, red letters: antigens are still unknown.
Green letters: tentative nomenclatures suggested by us

Figure 2. Classification and autoantigens in ABDs. In the schematic immunoblot (upper right panel), PV serum reacts with the 160 kDa Dsg1 and the 130 kDa Dsg3, BP serum reacts with the 230 kDa BP230 and the 180 kDa BP180 and paraneoplastic pemphigus (PNP) serum reacts with the 210 kDa envoplakin and the 190 kDa periplakin.

in EBA (Figures 1c, 2). In addition, many new target antigens have been identified by various biochemical and molecular biological methods in most ABDs, and the identification of new autoantigens suggested new disease entities (Figure 2) [20–30].

## Pathogenesis of ABDs (Figure 1d)

In almost all types of autoimmune diseases, autoantibodies to nuclear or organ-specific antigens are specific markers for diagnoses. However, in most diseases, particularly in various rheumatic diseases, the pathogenic roles of autoantibodies are unidentified. In sharp contrast, the pathogenic activity to produce blisters on the skin and mucous membranes is clearly demonstrated in most ABDs, mainly by various disease models described below [31,32].

This initial process of autoimmunity is probably common in distinct autoimmune diseases. There are many possible speculations for the mechanisms for loss of immune tolerance by abnormal immune regulations in the thymus (Figure 1d, right area). These include the abnormal negative selection in neonatal thymus, involving Aire protein, regulatory T cells (Tregs) and antigen expression by medullar thymic epithelial cells. Outside the thymus, immune tolerance disruption is explained by varieties of abnormal immune regulation involving Tregs, memory T cells, antigen-presenting cells, MHC class II, cytokines and regulatory B cells (Bregs) (Figure 1d, lower left area) [33,34]. This loss of tolerance develops only in genetically susceptible individuals, through products of both MHC and non-MHC genes [35–38].

Once autoantibodies are produced by unknown mechanisms, the ensuing immunological and inflammatory events for disease development are different among distinct autoimmune diseases. This is true also in ABDs. Pathogenesis of various ABDs has been investigated extensively using many distinct disease models, as described below, and each ABD results in blisters by a different pathway. Thus, in general, blisters in most pemphigus group diseases are developed by direct inhibition of cell adhesion or through abnormal signal transduction pathways after binding of autoantibodies to antigens. In contrast, various inflammatory events are necessary to develop skin lesions in various subepidermal ABDs (Figure 1d, upper left area).

## Disease models for ABDs

To best investigate the pathogenesis in any human diseases, various types of disease models, particularly animal models, are very useful tools [39,40]. This is also the case in various autoimmune diseases, including SLE, systemic sclerosis, rheumatic arthritis, autoimmune neural diseases or inflammatory bowel diseases.

For ABDs, Anhalt *et al* [41] first reported a disease model in which pemphigus skin lesions could be reproduced in neonatal mice injected with IgG fraction obtained from pemphigus sera. We also showed that anti-Dsg3 antibodies, affinity-purified from paraneoplastic pemphigus sera, could produce skin lesions in neonatal mice [42]. Subsequently, Liu *et al* developed a mouse model of BP and published a series of studies, which suggested that various factors were involved in the formation of blisters in BP, including complement, leukocytes, mast cells, proteinases and cytokines [43–45].

Due to immune tolerance, an active model for PV, in which wild-type mice were injected repeatedly with recombinant Dsg3, was not successful [46]. To overcome immune tolerance, $Dsg3^{-/-}$ mice were injected

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with recombinant Dsg3, resulting in development of skin lesions. In addition, the skin lesions also reproduced by transferring lymphocytes from Dsg3$^{-/-}$ mice to immune-deficient Rag-2$^{-/-}$ mice [46].

However, the situation of disease models in EBA is different from that in PV [47]. Sitaru *et al* first developed a so-called *ex vivo* model of EBA, in which skin sections on slides are induced to blister by incubation with autoantibodies and leukocytes [48,49]. Next, several groups developed passive mouse models of EBA, in which mice injected with rabbit antiserum raised against a recombinant COL7 NC1 domain undergo blister formation [50–52]. Finally, an active mouse model of EBA is also available, in which wild-type mice are immunized with recombinant protein containing the NC1 domain of murine COL7 [53]. It is not known why immune tolerance is overcome in EBA mouse model, but not in pemphigus models.

## Pathological features in EBA

While BP is the most common ABD in developed countries, EBA is rare and the prevalence of EBA is 5–10 times less than that of BP [54,55]. There is no racial predilection, although EBA shows slightly greater prevalence in females. Several studies reported an increased occurrence of HLA-DR in EBA [35–37]. HLA-DR2 is in general associated with hyperimmunity, even in other diseases.

In EBA, direct immunofluorescence demonstrates deposits of IgG and C3 at the BMZ, and indirect immunofluorescence detects circulating IgG anti-BMZ antibodies, which react with COL7 by immunoblotting of normal human dermal extracts and ELISA of recombinant proteins of NC1 and NC2 domains of COL7 [56–60].

EBA is usually extremely intractable, and the standard therapy with systemic steroids and immunosuppressive drugs is often unsatisfactory. Oral dapsone or oral colchicine can be effective. In addition, IVIG is often effective in intractable EBA patients [61]. IVIG seems to selectively decrease pathogenic autoantibodies, because circulating antibodies levels decreased quickly within 1–2 weeks after IVIG initiation [59].

Pathological mechanisms in EBA, as suggested by various disease models, are schematically summarized (Figure 1d, upper left area) [62–64]. After autoantibodies bind to COL7, a complement cascade is activated and the resulting C3a and C5a recruit leukocytes and mast cells, in addition to direct destructive actions at the BMZ [53,65]. Recruited leukocytes (mainly neutrophils) bind to the Fc domain of IgG antibodies through FcgRs expressed on the cell surfaces [48] (details are described below). Activated neutrophils produce elastase and gelatinase [66], which in turn activate metalloproteinases [67] that destroy extracellular matrices at the BMZ, resulting in loss of BMZ adhesion and subepidermal blister formation. The activated neutrophils also produce reactive oxygen species that also induce BMZ damage and accelerate subepidermal blister formation.

The inflammation that develops at the BMZ in lesional skin also activates keratinocytes and mast cells, which produce various cytokines [68]. These cytokines may play a role in promoting further inflammation, as well as induction of autoantibody production through activation of T and B cells.

## Previous studies of FcgRs

There are four murine FcgRs; FcgRI, FcgRIIB, FcgRIII and FcgRIV [69–71]. Binding of IgG induces cross-linking of FcgR chains on effector cells, which in turn activates various phosphorylation cascades, resulting in various immune responses, including antigen presentation and releases of inflammatory mediators. Proinflammatory cytokines also up-regulate activating FcgRs and down-regulate inhibitory FcgRIIB [71]. This is supported by the finding that mice deficient in common γ-chain of FcgRs are resistant to IgG-induced inflammation in various disease models [71]. Therefore, IVIG may ameliorate autoimmune response by changing the balance among various FcgRs [72].

In various autoimmune diseases, pathogenic effects were mediated through low-affinity FcgRIII and intermediate-affinity FcgRIV [73–75]. Thus, Fc fragments in IVIG preparations may down-regulate these activating FcgRs and prevent following tissue damage. In addition, IVIG was reported to increase expression of FcgRIIB on effector cells (macrophages). This assumption may be supported by the result that IVIG treatment did not protect disease induction in mice lacking FcgRIIB [76].

Specific ICAM-3 grabbing non-integrin-related 1 (SIGN-R1) has been identified as a receptor that up-regulates the expression of FcgRIIB in mice. Therefore, binding of IVIG preparation to SIGN-R1 may lead to up-regulation of FcgRIIB on effector cells [77].

## Involvement of FcgRs in EBA

As mentioned above, FcgRs are suggested to play an important role in the pathogenesis in EBA. In this issue of the *Journal of Pathology*, Kasperkiewicz *et al* elegantly investigated the role of different FcgRs, molecularly and functionally [78]. First, they analysed molecularly the altered gene expression profiles in EBA by weighted gene co-expression network analysis (WGCNA) [79], which followed DNA microarray study of mRNAs extracted from mice injected with anti-COL7 IgG and normal IgG [80]. This analysis identified 33 candidate genes, from which four genes (*FcgRIV, Ncf2, MMP-8* and *MMP-13*) were selected by close relationship to autoantibody-induced tissue injury found in previous EBA studies.

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Involvement of FcgRIV was further studied, because previous studies using various EBA models suggested that specific interaction between leukocytes (particularly neutrophils) and autoantibodies through FcgRs play an important role in the pathogenesis in EBA. However, the contribution of different FcgRs, including FcgRI, FcgRIII and FcgRIV, as well as inhibitory FcgRIIB, to EBA pathology had never before been investigated.

Kasperkiewicz *et al* [78] confirmed higher mRNA expression of *FcgRIV* by quantitative RT–PCR in experimental EBA mice induced by injection of anti-mouse COL7 antibody. In contrast, the mRNA level of inhibitory *FcgRIIB* was lower in EBA mice, thus the *FcgRIV* : *FcgRIIB* ratio increased to 1.56 from 0.13 in mice treated with normal rabbit antibody. To examine the relevance of this finding in human EBA patients, the expression of FcgRIIIA (a human counterpart of mouse FcgRIV) was examined by immunohistochemistry. FcgRIIIA was expressed in leukocytes infiltrated within the dermis, but not in leukocytes within the epidermis. FcgRIIIA expression did not increase in leukocytes in normal control skin.

Subsequently, to determine the role of each FcgR in EBA pathogenesis, functional analyses in a passive EBA animal model were performed using mice which were genetically deficient in different FcgRs. Mice lacking the inhibitory FcgRIIB, and given anti-mouse COL7 antibody, developed a significantly stronger clinical disease phenotype. In contrast, mice deficient in the common $\gamma$-chain of activating FcgRs showed no disease phenotype, even though these mice had equal amounts of autoantibodies, as shown by direct and indirect immunofluorescence.

The role of each activating FcgR was examined in more detail using other knockout mice. In *FcgRI* and *FcgRIII* knockout mice, as well as *FcgRI*/*FcgRIII* double knockout mice, no change in bullous lesions was seen after injection of anti-COL7 antibody. In contrast, *FcgRIV* knockout mice were completely protected from clinical and histological development of skin disease. Furthermore, injection of neutralizing anti-FcgRIV antibody in wild-type mice also protected against disease development. Finally, the protection against experimental EBA observed in mice deficient in common $\gamma$-chain was overcome by reconstitution of neutrophils from wild-type mice, and this further supported the key role of FcgRIV in experimental EBA.

From the results described above, Kasperkiewicz *et al* [78] convincingly concluded that skin lesions in experimental EBA mice were induced by neutrophils; effector cells bearing exclusively FcgRIV among the three activating FcgRs, while FcgRIIB had inhibitory action. This study has shown that experimental EBA is the first model in which disease is induced exclusively by FcgRIV, but not by other activating FcgRs. It was supposed that pathogenic antibodies first activate FcgRIV, which in turn binds to the Fc domain of anti-COL7 antibodies, resulting in disease induction.

This conclusion is supported by results in previous studies. Another study by the same authors indicated that in mice immunized with COL7, the expression of FcgRIV was higher in an EBA-susceptible strain than an EBA-resistant strain [65]. Results from an *ex vivo* EBA model suggested that IgG1 and IgG3 subclasses were capable of activating complement and induce blisters [49]. Furthermore, IgG1 and IgG3 subclasses also activated all FcgRs more strongly than IgG2 and IgG4 [72].

Most importantly, this study [78] suggested novel, targeted, therapies for EBA by inhibition of FcgRIV and activation of inhibitory FcgRIIB; eg injection of neutralizing antibody specific to FcgRIV. The monoclonal antibodies specific to molecules in FcgR signal pathway may also block the disease process [81]. IVIG preparations with higher ability in induction of inhibitory FcgRIIB or recombinant soluble FcgRIIB should be ideal therapeutic modalities.

## Perspectives and suggestions for the development of novel therapeutic strategies

FcgRs, particularly FcgRIV and FcgRIIB, are shown to play an important role in EBA pathogenesis. However, the real pathological mechanisms related to FcgRs have not been fully unravelled. The precise mechanism should be examined by future studies and lead to development of specific safer therapeutic modalities in EBA treatment. Possible therapeutic effects of IVIG should also be examined in this context because Fc fragments in IVIG preparations should interact with various FcRs.

## Acknowledgment

We thank Ms Hanako Tomita for secretarial work. This study was supported by Grants-in-Aid for Scientific Research and the Strategic Research Basis Formation Supporting Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by Health and Labour Sciences Research Grants and grants for Research on Measures for Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan.

## Author contributions

All authors contributed equally to the preparation of this commentary.

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