

SHORT COMMUNICATION

Immunosuppressive and Cytotoxic Cells in Invasive versus Non-invasive Bowen's Disease

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Bowen's disease (BD) is defined as epidermal carcinoma *in situ* that may progress to invasive BD retaining the cytological characteristics of BD (1). Previous reports suggested the contribution of tumour infiltrating leukocytes around the tumour, including regulatory T cells (Tregs), in organising the tumour microenvironment (2). In this report, we hypothesised that the profiles of tumour-infiltrating leukocytes might be correlated with the invasion of BD.

MATERIALS AND METHODS

We collected archival formalin-fixed paraffin-embedded skin specimens from 5 patients with BD, 5 patients with micro-invasive BD and patients with invasive BD treated in the Department of Dermatology at Tohoku University Graduate School of Medicine (Table S1¹). All diagnoses were made by typical clinical manifestations and histopathological examination. Two dermatologists counted and estimated by blind assessment the infiltrated lymphocytes. Antibodies (Abs) for immunohistochemical staining were then used, as described previously (3) (Table S11¹).

RESULTS

We performed immunohistochemical staining of CD163 (Fig. 1) as well as Foxp3 (Fig. S1¹). Only in invasive BD, dense CD163⁺ MΦ were detected throughout the dermis. In contrast to CD163⁺ cells, the number of Foxp3⁺ cells was significantly lower in invasive BD (Fig. 2).

Next, in order to compare the profiles of tumour-infiltrating CTLs between invasive, micro-invasive and non-invasive BD, we employed immunohistochemical staining for CD8 (Fig. S2A–C¹), granulysin (Fig. S2D–F) and TIA-1 (Fig. S2 G, I¹). There was no significant difference in the numbers of CD8⁺ cells, granulysin⁺ cells and TIA-1⁺ cells among these groups (see Fig. 2).

To further investigate the immunosuppression in the tumour microenvironment, we employed immunohistochemical staining for B7H1 (ProSci, Poway, CA) and MMP9. Interestingly, in non-invasive BD, B7H1 was strongly expressed on tumour cells (Fig. 3A). In contrast, B7H1 expressing cells were mainly observed on the tumour-infiltrating leukocytes around the tumour in micro-invasive (Fig. 3B) and invasive BD (Fig. 3C). Dense infiltration of MMP9⁺ cells was detected around

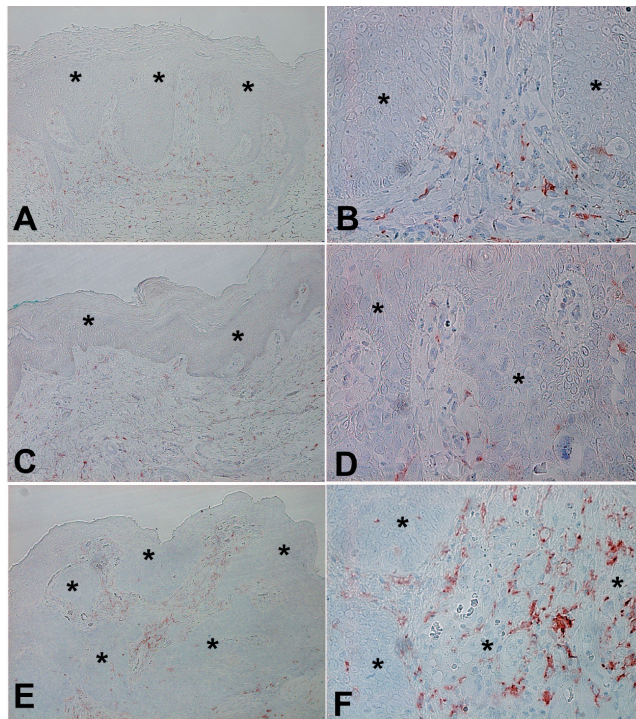


Fig. 1. CD163⁺ M2 MΦ in non-invasive, micro-invasive and invasive Bowen's disease (BD). Paraffin-embedded tissue samples from patients with non-invasive BD (A, B), micro-invasive BD (C, D) and invasive BD (E, F) were deparaffinised and stained with anti-CD163 Ab (A–F). Sections were developed with liquid permanent red. (CD163: staining for MΦ) (A, C, E: ×100; B, D, F: ×400). (Asterisk: Tumour site).

the tumour in invasive BD (Fig. 3D), while few MMP9⁺ cells were detected around the tumour in non-invasive BD (Fig. 3E) and micro-invasive BD (Fig. 3F).

DISCUSSION

Our results demonstrated dense infiltration of CD163⁺ MΦ throughout the dermis only in invasive BD. Recent reports suggested that the presence of macrophages correlates with therapy failure and poor prognosis in cancer patients (4, 5). More recently, it was reported that M2 MΦ cells have an important role in the production of thymus and activation-regulated chemokine, which leads to the induction of Tregs and Th2, and the composition of the immunosuppressive tumour microenvironment (6, 7). Therefore we hypothesised that there is correlation between the increasing numbers of M2MΦ and the numbers of Tregs. Unexpectedly, in contrast to the increasing

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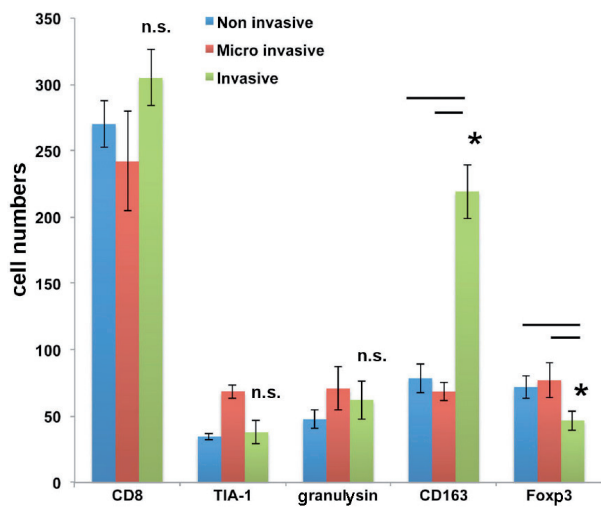


Fig. 2. Summary of the numbers of CD8⁺ cells, TIA-1⁺ cells, granulysin bearing cells, CD163⁺ cells and Foxp3⁺ cells in non-invasive, micro-invasive and invasive Bowen's disease. Five representative fields of each section were selected from both the epidermis and the dermis associated with dense dermal lymphoid infiltrate. The number of immunoreactive cells was counted using an ocular grid of 1 cm² at a magnification of $\times 400$. The data are expressed as the mean \pm SD of the numbers in each area. * $p < 0.05$.

number of M2M Φ , the number of Tregs is significantly decreased in invasive BD. This discrepancy might be explained by the expression of B7H1 in tumour and M2M Φ cells. Indeed, in our study, the high expression of

B7H1 in tumour cells is only prominent in non-invasive BD, which might be connected with the increased numbers of Tregs (8). Instead of B7H1 expression in tumour cells, invasive BD contains B7H1-expressing cells and MMP9-expressing cells around the tumour. As previously suggested, the expression of MMP9 in immunosuppressive macrophages in the tumour microenvironment contributed to tumour invasion and metastasis (3, 9, 10).

To further investigate the immunological environment of BD, we investigated the population of cytotoxic T cells, focusing on CD8, granulysin and TIA-1. CD8 is a classical common marker for cytotoxic T cells, while granulysin and TIA-1 have been reported to be functional markers for cytotoxic T cells, and correlate with the prognosis of cancer patients (11–13). In contrast to immunosuppressive cells, there was no significant difference in tumour infiltrating cytotoxic T cells at any stage of BD, suggesting that the immunological background of the tumour microenvironment in BD might be determined by immunosuppressive cells such as M2 M Φ and Tregs. Since we did not directly assess the suppressive function of these infiltrating M2 M Φ or cytotoxic T cells, further analysis of the mechanisms underlying this phenomenon will be necessary to confirm our limited observation.

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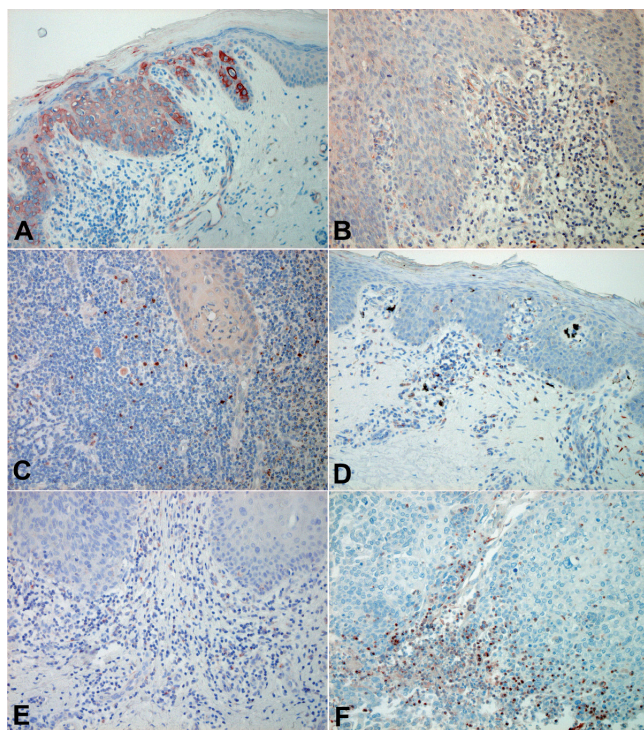


Fig. 3. The expression of B7H1 and MMP9 in non-invasive, micro-invasive and invasive Bowen's disease. Paraffin-embedded tissue samples from patients with non-invasive BD (A, D), micro-invasive BD (B, E) and invasive BD (C, F) were deparaffinised and stained with anti-B7H1 Ab (A–C) or with anti-MMP9 AB (D–F). Sections were developed with liquid permanent red. (A–F: original manifestation $\times 200$).

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