Peritumoral Inflammation in Basal Cell Carcinoma: Fundamentals, Clinical Significance, and Changes After Topical Imiquimod Therapy

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Peritumoral inflammation surrounding basal cell carcinoma (BCC) is complex and may provide valuable information regarding host response, which is not entirely successful at eradicating or containing tumor progression, and successful immunologic response, which may lead to therapeutic success. Therapies for BCC, such as intralesional interferon and topical imiquimod, alter the nature and intensity of peritumoral inflammation. This article reviews current understanding of cell types and their suggested functions in peritumoral inflammation surrounding BCC and discusses the changes induced by topical imiquimod use.

The pathogenesis of basal cell carcinoma (BCC) results from a multifactorial process that involves environmental risk factors, genomic alterations (eg, hedgehog signaling pathway disruption, alteration of tumor suppression genes), deviations in cell proliferation, apoptosis, immune surveillance, and differentiation.[1] Although the most frequent therapeutic approaches for BCC are surgical excision and curettage and electrodesiccation, recognized treatment modalities include cryosurgery, photodynamic therapy, and use of immune response modulators, such as intralesional interferon (IFN) and topical imiquimod.[2] Compared with surgery, the appropriate use of pharmacologic agents for properly selected BCCs may allow for potentially lower morbidity and improved tissue preservation.[3]

The therapeutic activity of topical imiquimod involves stimulation of innate and cell-mediated immune response.[3,4] With the development and use of immunomodulators such as interferon and imiquimod, recent studies have attempted to examine, both quantitatively and functionally, the peritumoral infiltrate surrounding BCC.[4-7] This article will examine the literature pertaining to the peritumoral cellular infiltrate associated with untreated BCCs and in BCCs after treatment with topical imiquimod. Although each cell type within the infiltrate surrounding BCC is characterized by specific cellular antigens, expression of antigens among the various cells can overlap. Therefore, it is primarily the combination of cellular markers that designates a specific cell type. Understanding the

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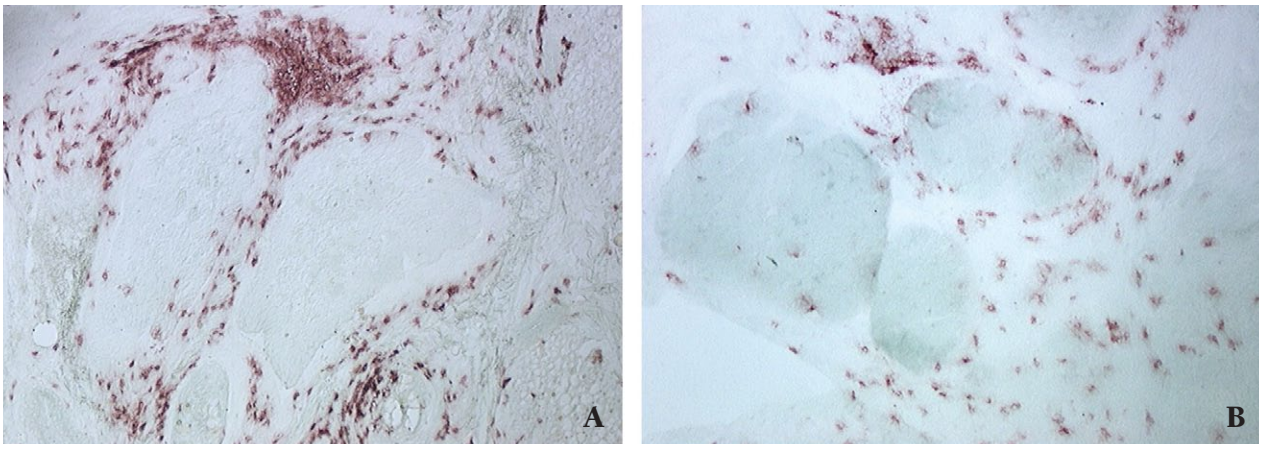

Figure 1. Nodular basal cell carcinoma with aggregates of $CD3^+$ (A) and $CD8^+$ (B) T cells in peritumoral areas (light green, original magnification ×10).

peritumoral infiltrate and alterations that occur after treatment for BCC may facilitate predicting recurrence rates as well as the development of additional immune-based therapies for BCC.

IMMUNE SYSTEM AND TUMOR BIOLOGY

To better understand the effects of imiquimod on the inflammatory cell infiltrate surrounding BCC, the phenotypic cells associated with untreated BCC are examined. The peritumoral infiltrate seen in association with untreated BCC involves both the innate and adaptive immune systems. The innate immune system has direct effects on tumor cells. Innate immune cells associated with BCC include dendritic cell (DC) subtypes (eg, Langerhans, myeloid, and plasmacytoid cells), dermal mast cells, macrophages, neutrophils, and natural killer (NK) cells, all of which release cytokines and chemotactic proteins, which recruit additional cells. Adaptive immune cells associated with BCC predominantly include T lymphocytes, which are capable of penetrating and infiltrating the peritumoral region (Figure 1). Effective antitumor immunity involves the interaction and bridging between the innate and adaptive immune responses.

IMIQUIMOD: MECHANISM OF ACTION

Immune system activation appears to correlate with successful eradication of BCC as well as of other neoplasms treated topically with imiquimod, including actinic keratosis and squamous cell carcinoma (SCC) in situ.[7,8] Although the exact mechanisms by which imiquimod induces tumor regression are not completely understood, multiple studies have examined the effect of imiquimod on inflammatory response.[9-11] Upon binding to its receptor, imiquimod, a Toll-like receptor 7 (TLR7) agonist, upregulates production of inflammatory cytokines, such as IFN-α, tumor necrosis factor alfa (TNF-α), and interleukin-12 (IL-12) (promoting cell-mediated immune response) and results in indirect activation of nuclear factor kappa B (NF-κβ), an immune-response–regulating transcription factor; imiquimod also appears to exhibit weak TLR8 agonist properties.[12] Ultimately, imiquimod enhances the interaction between the innate and adaptive immune responses, as binding of TLR agonists results in indirect stimulation of the NF-κβ signaling pathway. NF-κβ and IFN-α are considered key links between the innate and adaptive immune systems.[13,14] Furthermore, imiquimod reduces the expression of Bcl-2, an antiapoptotic protein, and also has been shown to inhibit angiogenesis.[11]

POTENTIAL IMPACT OF BCC SUBTYPE

BCCs are commonly subdivided according to their histologic pattern. There are various histologic BCC subtypes—superficial, nodular, micronodular, infiltrative, and morpheic. One recent study identifies significant correlations between BCC subtypes with inflammatory and stromal alterations.[15] It was demonstrated that superficial BCC consisting of features characteristic of old regression was associated with moderate to dense peritumoral lymphocytic infiltrate, and infiltrative and morpheic BCC was associated with fibrosing tumor stroma. In contrast to both superficial BCC with changes of regression and infiltrative and morpheic subtypes, micronodular BCC had loss of both host inflammatory and stromal tumor responses.[15] It may be concluded that distinct epithelial, stromal, and inflammatory patterns correlate with BCC histologic subtype and tumor progression. A better understanding of the characteristics of the cellular infiltrates surrounding BCC and patterns of stromal response may facilitate

development of immune-based treatments for BCC and more directed modulation of the biologic behavior of the tumor (ie, the degree of locally aggressive behavior).

PERITUMORAL INFLAMMATION: ANALYSIS OF CELL TYPES

Dendritic Cells

DCs comprise a moderate proportion of the infiltrate surrounding BCC. These cells serve as antigen-presenting cells (APCs) and are therefore necessary for immune surveillance and tumor recognition by the immune system. There are various subsets of DCs, which are potent activators of the immune response system.[16] BCC is associated with DC subsets, such as Langerhans cells and myeloid and plasmacytoid DCs. These DC types are identified by specific cell surface markers, such as CD1a, CD11c, and CD123, respectively (Table 1). Myeloid DCs (mDCs) produce TNF-α and inducible nitric oxide synthtase, thereby augmenting innate immune defense. Plasmacytoid DCs (pDCs) produce IFN-α and are hypothesized to be directly involved in T-cell activation.[16]

Recently, it has been proposed that TLR expression may identify DC subsets, such as mDCs and pDCs, signifying that these cells not only serve as APCs but also play a specific role in the effector phase of immune response. TLR7/8 induced the expression of additional cell surface antigens on mDCs and pDCs.[17] Although Langerhans cells, mDCs, and pDCs are associated with therapy-naïve BCC, imiquimod significantly increased the number of only mDCs and pDCs.[10] We will revisit specifically how DCs were stimulated by TLR7/8 to serve as effector cells.

Mast Cells

Mast cells, in addition to DCs, contribute to the BCC peritumoral infiltrate. BCC has been characterized by increased numbers of mast cells in peritumoral stroma.[18] TLR-mediated mast cell activation results in a milieu of cytokine and leukotriene release. These mast cell products function to initiate and regulate immune response patterns and generate adaptive immune responses. Mast cell degranulation induces factor XIIIa expression[19] and glycoprotein 1b-α on dermal dendrocytes, which may play a role in immune response modulation.[20] It has been postulated that dermal dendrocytes expressing glycoprotein 1b-α and factor XIIIa may potentially interact with mast cells and exhibit a role in angiogenesis.[18,21] It may be presumed that more aggressive forms of BCC may have increased the numbers of dermal dendrocytes as a result of being associated with angiogenesis; however, there is currently no correlation between the various aggressive and nonaggressive histologic subtypes of BCC and the density of dermal dendrocytes. The role of mast cell and dermal dendrocytes in BCC remains elusive. BCCs are also populated by benign melanocytes, but as with mast cells and dermal dendrocytes, the significance of the melanocytes is still unknown.[22]

TABLE 1

Peritumoral Cells Associated With Basal Cell Carcinoma*

Cell Type	Characteristic Surface Antigens
T helper	$CD4^+CD3^+$
Cytotoxic T	$CD8^+CD3^+$
T regulatory	$CD4^+CD25^+FOXP3^+$
Myeloid DC	$CD11c^+HLADR^+$ or $BDCA\text{-}1^+DEC205^+CD11b^+$
Plasmacytoid DC	$CD123^+$(IL-3 receptor) $CD45RA^+$ or $BDCA\text{-}2^+HLADR^+$
Dermal dendrocytes	Factor $XIIIA^+Gp1ba^+$
Langerhans	$CD1a^+Langerin^+$
Phagocytes	$CD14^+$
Neutrophil	$CD15^+$
Natural killer	$CD56^+CD94^+$
Mast	$c\text{-}Kit^+$
B cell	$CD19^+$

Abbreviations: T, T lymphocyte; B, B lymphocyte; DC, dendritic cell; Gp, glycoprotein; IL, interleukin.

*Surface antigens have overlapped expression. A combination of surface antigens defines a specific cell type.

EFFECT OF IMIQUIMOD ON PERITUMORAL CELLULAR INFILTRATE

Examination of untreated BCCs has demonstrated that the peritumoral cellular infiltrate is heavily comprised of cytotoxic and helper T cells, with few or no natural NK cells.[10,23] Interestingly, imiquimod therapy dramatically increased the number of NK cells associated with the peritumoral infiltrate of BCC.[9,10] NK cells can be identified by the following cell surface markers, in addition to a few others: CD16, CD56, and CD57.[24] These cells are associated with both antibody-dependent and antibody-specific NK cellular cytotoxicity and also produce IFN-γ.[25] The presence of NK cells may help to explain BCC regression induced by both IFN and imiquimod.

Barnetson et al[26] also demonstrated that imiquimod increased the cellular infiltrate of BCC. After treatment with topical imiquimod, there was early appearance of $CD4^+$ cells, activated DCs ($CD11c^+$ $CD86^+$), and macrophages (Mac 387). The later infiltrate included $CD8^+$ cells. Importantly, all the cell types were juxtatumoral. The results of this analysis suggest that a cell-mediated immune response appears to be at least partially responsible for imiquimod-induced regression of BCC; BCC treated by placebo demonstrated no significant cell infiltration.

Application of topical imiquimod, although largely affecting mDC, pDC, and T-cell migration, appeared to only slightly modify the density of dermal dendrocytes in one study.[5] Quatresooz and Piérard[5] suggested that pretreatment density of dermal dendrocytes may affect the treatment outcome after application of topical imiquimod. BCC responsive to imiquimod contained many dermal dendrocytes prior to treatment whereas refractory lesions had fewer dermal dendrocytes.[5]

PATTERNS OF CELLULAR INFILTRATE WITH SPONTANEOUS BCC REGRESSION

Although believed to be uncommon, partial spontaneous regression of BCC has been reported.[27,28] One study examined the density of the cellular infiltrate within both regressing and nonregressing BCC.[29] In contrast to nonregressing tumors, regressing tumors exhibited markedly increased numbers of $CD3^+$ and $CD4^+$ T cells.[29] It is important to recognize that the increased density of $CD4^+$ cells is not comprised solely of T cells. $CD4^+$ antigen is also expressed on DCs and therefore suggests that both DC and T cells are involved in tumor regression.[30] Few B lymphocytes are seen in both regressing[29] and nonregressing BCC.[10]

PERITUMORAL INFILTRATES AND ANTITUMOR ACTIVITY

T regulatory cells (Tregs) have been demonstrated in the peritumoral infiltrate of BCC (Figure 2).[30] Tregs are a type of T cell that appear to control immunologic responses that appear to suppress APCs, certain T cells, and NK cells.[31-33] Examination of the effects of Treg depletion in human malignancies demonstrated increased T-cell activation and tumor regression, suggesting that Tregs suppress antitumor responses.[31] Although there are varying subsets of Tregs, the classic Tregs are phenotypically designated as $CD4^+CD25^+FOXP3^+$ T cells. All 3 markers are necessary to satisfy the definition of a Treg since CD4 antigen can also be located on T helper cells and DCs and CD25 can be considered a T-lymphocyte activation marker. TLR8 ligand binding mediated the reversal of $CD4^+$ Treg function and has been shown to enhance antitumor immunity in mice.[34] Accordingly, imiquimod, primarily a TLR7 agonist that also appears to exhibit weak TLR8 agonist properties, may be involved in enhancing antitumor immunity in humans in a similar fashion.

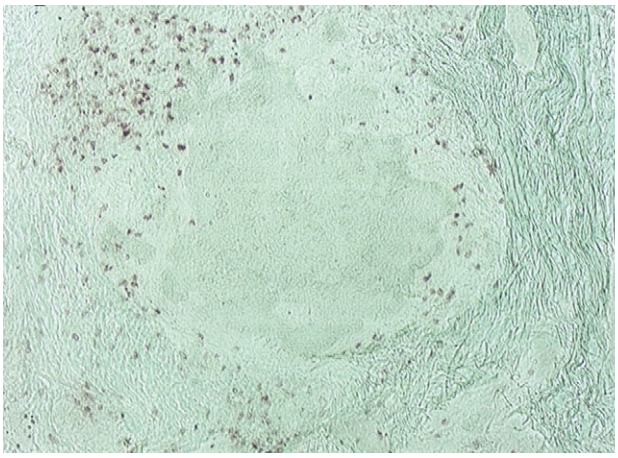

Figure 2. $Foxp3^+$ T cells in peritumoral areas of nodular basal cell carcinoma (light green, original magnification ×10).

ROLE OF DENDRITIC CELLS AS EFFECTOR CELLS

Stary et al[10] compared the peritumoral infiltrate in both naïve and imiquimod-treated BCCs. Imiquimod induced both quantitative and qualitative alterations of the inflammatory infiltrate within the peritumoral stroma. Imiquimod-treated BCCs were associated with a dramatic increase of $CD8^+$ and $CD4^+$ T cells, NKs, neutrophils, and pDCs, and a moderate increase in mDCs (Table 2). In contrast, the numbers of B cells, eosinophils, and mast cells did not change significantly.

In addition to the quantitative changes of immune cells, imiquimod-treated BCC, in contrast to pretreated specimens, showed perforin and granzyme B expressed on mDCs ($CD11c/HLADR^-$) and TNF-related apoptosis-inducing ligand (TRAIL) on pDCs. This is clinically relevant because perforin and granzyme B are linked to cytotoxicity, and mDCs, which express granzyme and perforin, coexpress the antitumor proinflammatory mediators TNF-α and inducible nitric oxide synthase. As a result, these cellular changes induced by imiquimod appear to be involved with innate anticancer effector function. Furthermore, following imiquimod therapy, peritumoral pDCs produced IFN-α, which induces cytotoxic molecules on NK cells and T cells, thus demonstrating that pDCs may indirectly contribute to tumor cell eradication.

The development of cytotoxic DCs as tools in anticancer immunotherapy is significant not only in the induction of an immune response but also in the effector

TABLE 2

Peritumoral Cells in Which Imiquimod Induces Sizable Increases of Infiltrate in Basal Cell Carcinoma

Cell Type	Surface Antigens
Cytotoxic T	$CD8^+CD3^+$
T helper	$CD4^+CD3^+$
Natural killer	$CD56^+CD94^+$
Plasmacytoid DC	$CD123^+CD45RA^+$
Myeloid DC	$CD11c^+HLADR^+$
Neutrophil	$CD15^+HLADR^-$

Abbreviations: T, T lymphocyte; DC, dendritic cell.

phase of the immune response. Stary et al[10] examined the functional relevance of these imiquimod-induced perforin-positive and granzyme B–positive mDCs and the TRAIL-positive pDCs with TLR7/8. Both cell types (the perforin-positive granzyme B–positive mDCs and the TRAIL-positive pDCs) displayed direct cytotoxicity against tumor cell lines.

OTHER CONSIDERATIONS

The aforementioned studies predominantly examined the peritumoral cellular infiltrate in nonimmunocompromised individuals. Interestingly, immunosuppressed organ transplant recipients are at greater risk for developing cancer, including SCC, yet increased incidence of BCC has not been described in organ transplantation patients. It may therefore be of interest to formally compare the infiltrate of BCC and SCC among this patient population.

SUMMARY

Peritumoral infiltrates associated with BCC are associated with infiltration with $CD4^+$ cells, $CD8^+$ cells, Tregs, pDCs, mDCs, dermal dendrocytes, Langerhans cells, NK cells, and macrophages. Imiquimod primarily increases the numbers of T lymphocytes, NK cells, pDCs, and mDCs, responses that appear to correlate with antitumor activity. In studies evaluating use for BCC, imiquimod enhances antitumor immunologic response, resulting in histologic and clinical clearance of BCC.

The relationship between tumor cells and tumor-infiltrating DC subtypes is complex, with some DCs appearing to exhibit important roles in antigen recognition and effector cell activity. Progressing tumors are effective at limiting immune response and escaping recognition by the host during immune surveillance. Increased understanding of DC and T-lymphocyte phenotype and function are likely to facilitate the development of additional pharmacologic agents that effectively modulate specific immune response patterns and eradicate tumors. As with imiquimod, such agents may serve to provide both primary and adjunctive therapeutic roles.

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