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Original contribution

A morphological and immunophenotypic map of the immune response in Merkel cell carcinoma ☆

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Summary The susceptibility of Merkel cell carcinoma to the host immune response has prompted a search for effective immunotherapy. CD8-positive T lymphocytes are considered key effectors of this response, but the cellular infiltrates also harbor tumor-protective agents. By developing a comprehensive morphological and immunophenotypic map of tumor-infiltrating lymphocytes (TILS) in Merkel cell carcinoma, we aimed to establish a useful template for future studies. Twenty-two cases (mean age, 79 years [range, 52-95]; male-female ratio, 10:12) were studied. TILS were categorized as brisk (7), nonbrisk (9), and absent(6). Merkel cell polyomavirus (MCPyV)–positive (16) and –negative (6) cases were included, as were those with pure (18) and combined (4) morphologies. One MCPyV+ case had undergone spontaneous regression. Immunohistochemical markers included CD3, CD4, CD8, CD20, CD68, FoxP3, PD-1, and CD123. Statistical analysis used Fisher exact tests and Spearman correlations. There was a significant correlation between brisk TILs and MCPyV+ status ($P = .025$). CD8+ T lymphocytes predominated, were present in significantly higher proportions in brisk infiltrates ($P = .003$), and showed a significant predilection for the intratumoral environment ($P = .003$). Immune inhibitors including T regulatory cells (FOXP3+) and PD-1+ "exhausted" immunocytes were present in lower proportions. Our findings support (1) the link between a brisk immune response and MCPyV positivity, (2) the supremacy of CD8+ cells in effecting immunity, and (3) the incorporation of immune inhibitors within the global infiltrate. Efforts to therapeutically arm the "effectors" and disarm the "detractors" are well focused. These will likely have the greatest impact on MCPyV-positive cases.

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1. Introduction

Attempts to favorably exploit the host immune response to cancer have been a major focus of interest in oncology in recent years [1–3]. Therapeutic interventions which augment effectors of immunity and/or disable immune checkpoints that block the antitumoral responses have resulted in improved survival in the setting of several malignancies. Merkel cell carcinoma (MCC), a lethal primary cutaneous neuroendocrine carcinoma of the elderly, is known to be susceptible to immune modulation. Its higher incidence and worse prognosis in immunocompromised individuals [4], its known viral pathogenesis (Merkel cell polyomavirus [MCPyV]) in the majority of cases [5], and its capacity to undergo complete spontaneous regression [6] all testify to its immune susceptibility.

The absence of effective chemotherapy for advanced MCC is a strong stimulus to explore the potential benefits of immunotherapy. Earlier reports of favorable survival in patients whose tumors harbored abundant tumor-infiltrating lymphocytes (TILS) [7], particularly in the setting of MCPyV positivity [7,8], and recent publications highlighting the independent favorable prognostic implications of high intratumoral CD8-positive lymphocyte scores lend substance to this investigative approach [9,10]. Moreover, immune-blocking mechanisms effected by T regulatory cells (Tregs) [11] and by the programmed cell death–1 receptor/ligand (PD-1/PD-L1) pathway have been identified in MCCs [12,13]. Such insights offer the potential to improve patient outcome by selective immunotherapy. The previous specifically targeted studies have provided invaluable information as outlined above. Our aim, in developing a morphological and immunotypic map of the immune response in MCC, was to provide a global semiquantitative and topographic appreciation of the relevant agonists and antagonists involved in this process.

2. Materials and methods

Ethical approval to pursue the project was obtained from the Capital District Health Authority Research Ethics Board. The study population consisted of a subset of a regional cohort of cases of MCC, which has formed the basis of previous publications [14–17]. Twenty-two patients from Maritime Canada whose tumors were diagnosed between 1999 and 2013 were included. Case selection was focused on inclusion of MCCs with (1) TILS of varying density, (2) MCPyV-positive and MCPyV-negative profiles, (3) pure and combined morphologies, and (4) adequate tissue for evaluation. The decision to restrict the number of cases studied was governed by resource availability and the primary objective of generating descriptive rather than epidemiologic data. The diagnosis of MCC in the global cohort had previously been verified on the basis of morphological features and immunohistochemical expression of at least one neuroendocrine marker in addition to paranuclear dot-like expression of cytokeratin 20 or a cytokeratin cocktail [16]. Tumor type had also been previously categorized as pure or combined, the latter including lesions with neuroendocrine and other carcinomatous or sarcomatous elements. Demographic and clinical data were derived from records accrued on the global cohort. In the absence of adequate information on disease-specific survival, outcome was recorded as overall survival.

Paraffin-embedded blocks from 19 resection specimens (of which 15 were primary excisions without prior biopsies) and 3 biopsies were available for the study. One representative section stained with hematoxylin and eosin (H&E), and 14-16 blank slides on charged slides were sent to colleagues at the Research Unit of Dermatopathology in Graz, Austria. The authors there, blinded to specific details of the cases, reviewed the H&E-stained sections and quantified the TILS as brisk (B), nonbrisk (NB), or absent (A) in accordance with guidelines for MCC [18]. The presence of plasma cells in the infiltrates was recorded semiquantitatively as "0" (none), "+" (few), or "++" (many).

The same authors conducted immunohistochemical studies on the cases and evaluated them in a systematic manner. The panel included stains for MCPyV (Santa Cruz, Heidelberg, Germany), CD3 (T lymphocytes) (Novocastra-Leica Mikrosysteme, Vienna, Austria), CD20 (B lymphocytes) (Dako Austria, Vienna, Austria), CD68 (macrophages) (Dako), CD8 (suppressor/cytotoxic T lymphocytes) (Dako), CD4 (helper T lymphocytes and some macrophages) (Novocastra), FOXP3 (Tregs) (Serotec, Oxford, UK), PD-1 (T lymphocytes with "T-cell exhaustion" marker) (Abcam, Cambridge, UK), and CD123 (plasmacytoid dendritic cells [PDCs]) (BD Biosciences, Schwechat, Austria). All sections were stained with a standard immunohistochemical technique on automated immunostainers (Dako, Dakopatts). The CD3 stain was used to corroborate quantification of TILS (B, NB, or A) on routine microscopy. The proportions of different cell types in the total infiltrate (%TI) and their topographic distributions were recorded. The CD4/CD8 ratios in the intratumoral and peritumoral environments were documented. The above evaluations, incorporating cytomorphological and immunohistochemical detail, were conducted independently by 2 investigators (G. F. and L. C.), and discrepancies were resolved by consensus. The findings were then correlated with clinical, demographic, and pathological variables.

Demographic, clinical, and pathological characteristics were summarized as medians and interquartile ranges (IQRs) for continuous data and frequencies with percentages for categorical data. The percentages of different phenotypic cells as components of the total infiltrate in cases with B and NB infiltrates were summarized as medians and IQRs. Mortality, pure morphology, and MCPyV+ status were compared across TILS densities using Fisher's exact test. The intratumoral and peritumoral CD4/CD8 ratios were compared as (1) the percentage expressing a ratio <1 and (2) the median ratios using Fisher's exact and Wilcoxon rank-sum tests, respectively. The interrelationships between the proportions of different immunophenotypes in the infiltrates were assessed using Spearman correlations.