Expression of Activation Antigens by T Cells Infiltrating Basal Cell Carcinomas

FRANCISCO J. GUILLEN, M.D.,* CALVIN L. DAY, JR., M.D.,† AND GEORGE F. MURPHY, M.D.

Department of Pathology, Brigham and Women's Hospital, and Dermatopathology Unit, Harvard Medical School, Boston, Massachusetts, and The Chemosurgery Unit, New York University Medical Center, New York, New York, U.S.A.

The association of T lymphocytes and dendritic cells with the stromal mononuclear cell response to basal cell carcinomas has led to speculation that cellular immunity may, in part, regulate the growth and development of this neoplasm. It has not been established, however, whether these T cells are functionally competent, or simply coincidental bystanders. We examined the immunologic phenotypes of mononuclear cells in 32 lesions of basal cell carcinoma obtained from 26 patients. The majority of infiltrating mononuclear cells were T cells that were equally distributed between the helper/inducer (Leu 3a+ and cytotoxic/suppressor (Leu 2a+) subtypes; a minority of cells were dendritic and expressed Leu 6 antigen. Virtually all T cells and dendritic cells were HLA-DR+, and many (≥30%) of the T cells expressed antigens consistent with stages of ongoing activation (T9, T10). TS2/7, a novel monoclonal antibody recently documented to identify activation-specific subcomponents of 210/165/130 kD glycoprotein complex present on the surface of mitogen- or alloantigen-stimulated human T cells, was also used. Greater than 50% of the T cells observed were TS2/7+. These observations provide information about site immunomorphologic evidence of stromal T cell activation in association with basal cell carcinomas, and suggest a role for active and ongoing cellular immune mechanisms as a determinant of local biological behavior of this neoplasm.

A number of recent studies have focused on the in situ association of various subsets of lymphocytes, identified by immunologic methods, with cutaneous alterations typical of a variety of inflammatory dermatoses and cutaneous neoplasms [1-5]. The immunologic phenotypes of these cells (e.g., helper/inducer subset) provide little information, however, concerning their actual-state functional competency [6-9]. Recently, monoclonal antibodies have been developed that define glycoproteins essentially restricted, at extrathymic sites, to activated T cells [10]. Detection of these activation antigens may be expected to provide insight into the functional capacity and responsiveness of mononuclear cells infiltrating the skin.

The role of local cellular immunity in basal cell carcinoma has been the subject of several recent investigations [11-14]. Mononuclear cell infiltrates associated with these neoplasms are comprised predominantly of T cells [11] that are associated with Langerhans cells in a manner akin to that observed in delayed-hypersensitivity responses [12,13]. Indeed, it has been suggested that tumor-specific antigens may be instrumental in inciting these cellular infiltrates [14]. These studies have been limited, however, by inclusion of relatively small patient populations. Moreover, detailed assessment of the state of functional activation of these infiltrating T cells has not been performed.

In order to determine whether T cells associated with basal cell carcinomas express activation antigens, we studied 32 lesions from 26 patients with a panel of monoclonal antibodies, including OKT9, OKT10, and TS2/7. OKT9 recognizes the transferrin receptor [15,16] of proliferating cells, and has been shown to identify T cells in early phases of activation, before the onset of DNA synthesis [6]. OKT10 recognizes a 46 kD antigen [15,17] that is expressed during the intermediate stage of T-cell activation [6]. TS2/7 is a newly described monoclonal antibody that reacts only with activation-specific subcomponents of a glycoprotein complex (A-1A5) found on the surfaces of mitogen- or alloantigen-stimulated T cells [10,18]. Our data suggest that many T cells characteristic of the inflammatory infiltrates of basal cell carcinomas express activation antigens and therefore are likely to be participants in an ongoing local cutaneous immune response. The potential significance of these observations is discussed in regard to the indolent biologic behavior of these neoplasms is discussed.

Patient Population

Thirty-two excisions were obtained from 26 patients who presented for dermatologic evaluation as outpatients. All specimens were obtained from sun-exposed sites and all patients had historical evidence of long-standing sun exposure. No patients had historical abnormalities, evidence of basal cell nevus syndrome, or overt immunologic abnormalities. Detailed clinical questionnaires were maintained and included information relevant to skin type, sun exposure, and biologic aggressiveness of lesions (size, ulceration, rapidity of growth). Clinical photographs were obtained in the majority of patients. Specimens were frozen immediately in OCT (Optimal Cutting Temperature; Lab-Tek, Division of Miles Laboratories, Naperville, Illinois) and subsequently sectioned on a cryostat for diagnostic evaluation and immunohistochemical staining.

Monoclonal Antibodies

The monoclonal antibodies Leu 2a, Leu 3a, and anti-HLA-DR (Becton Dickinson, Mountain View, California) were used for routine immunophenotyping. Leu 2a reacts with T lymphocytes of the cytotoxic/suppressor subset. Leu 3a reacts with T lymphocytes of the helper/inducer subset. Leu 6 reacts with Langerhans cells and indeterminate cells [19,20] but not with peripheral T cells, and anti-HLA-DR reacts with the nonpolymorphic region of human HLA-like antigens. In addition, OKT9, OKT10 (Ortho Pharmaceutical, Raritan, New Jersey), and TS2/7 (courtesy of Steven J. Burakoff, M.D.), were used to assess stages of T-cell activation in 7 specimens. OKT9 reacts with the transferrin receptor [15,16] and is first observed during early stages of T-cell activation [6]. OKT10 identifies T cells in intermediate stages of proliferation and activation [6], and TS2/7 [10] specifically identifies mitogen- and alloantigen-stimulated T cells during late stages of their activation.
Immunohistochemistry

The technique employed primary monoclonal antibodies, a biotinylated secondary antibody, and an avidin-biotin-horseradish peroxidase complex (Vector Laboratories Inc., Burlingame, California). This technique has been described previously in detail [21]. All sections were stained with 3,3'-diaminobenzidine (DAB) (Sigma, St. Louis, Missouri) for 60 s and rinsed in phosphate-buffered saline to terminate the staining reaction. Sections were counterstained lightly with methyl green prior to observation by light microscopy.

Quantification of Positive Cells

Because some variability resulted from regional differences in adjacent sections of tissue, relative cell numbers were determined semiquantitatively as percentages of total mononuclear cell number. Enumeration of positively labeled cells were facilitated by the use of an ocular grid micrometer.

RESULTS

Clinically, 5 cases were biologically aggressive (large, rapidly growing lesions). There was no correlation, however, between histology, including mitotic rate, or immunohistochemical staining patterns and clinical parameters of biologic aggressiveness or of duration of lesions. Histologic evaluation revealed all lesions to be invasive basal cell carcinomas. Although some also showed associated multiple superficial foci within the epidermis, sclerosing or anaplastic (metatypical) variants were not observed.

The majority (>90%) of mononuclear cells in the stromal infiltrates were reactive with either anti-Leu 2a or anti-Leu 3a antibodies. On the average, approximately 50% were Leu 2a+ and 50% were Leu 3a+, with a range of 40–60% in individual cases (Fig 1A,B). All of these cells expressed HLA-DR antigen (Fig 1C), as determined by quantitative evaluation of adjacent sections. T cells were intimately associated with tumor cells comprising both invasive nests and multiple superficial foci. Infiltration of tumor nests by T cells was focally observed, particularly by cells of the Leu 2a+ phenotype. Leu 6+/HLA-DR+ dendritic cells were present in normal to apparently increased numbers within the perilesional epidermis. Similar numbers of dendritic cells were present within the tumor stroma and the tumor nests (Fig 1D). The dendrites of these cells frequently were elaborate and surrounded adjacent epithelial cells, forming an apparent intercellular staining pattern focally within the epidermis and tumor nests (Fig 1E).

Discrete foci of OKT9- and OKT10-positive mononuclear cells were observed in the stromal infiltrates. These cells often comprised >30% of the mononuclear cells in these regions and were associated with tumor nests (Fig 2A,C), but not with perilesional epidermis. Basal cell carcinoma cells at the periphery of invasive nests also stained with OKT9 (Fig 2B). Variable staining of mononuclear cells with TS2/7 was observed, and TS2/7+ cells comprised >50% of numerous stromal foci (Fig 2D). In addition, staining of vessel walls and smooth muscle, as previously reported [7], was also observed. This pattern of reactivity facilitated the morphologic definition of the vascular stroma characteristic of basal cell carcinoma and of adjacent normal skin (report in preparation).

DISCUSSION

We have demonstrated that subpopulations of stromal T cells associated with basal cell carcinomas express antigens indicative of various stages of their functional activation. These data extend previous observations of immunologic phenotypes of mononuclear cells infiltrating these neoplasms [11,14], and provide additional evidence for an ongoing local immune response.

Basal cell carcinomas are characterized by relatively indolent growth, and only rarely metastasize [22]. Aggressive biologic behavior appears to be related to size and growth characteristics of lesions, or defects in immunologic status in certain individuals [23,24]. Defective local immunosurveillance has been suggested to play a role in the genesis of these tumors in young individuals prone to multiple lesions [25]. In addition, morphologic and immunohistochemical studies have documented the association of activated lymphoid cells with cutaneous tumors.

![Fig 1. A, Leu 3a; B, Leu 2a; and C, HLA-DR-positive mononuclear cells associated with basal cell carcinoma (BCC). Note migration of Leu 2a+ cells into tumor (B, arrowhead). D, Dendritic Leu 6+ cells within tumor stroma and infiltrating tumor nests (arrowheads). The highly dendritic nature of these cells focally produce an intercellular staining pattern (E). (A–C, × 400; D, × 800; E, × 1000).](image-url)
that may be characterized by clinical regression, such as malignant melanoma [4,26]. It is reasonable to hypothesize that if T cells are involved in a chronic response to local cutaneous (tumor-related) antigens, they would cycle asynchronously at various stages of functional activation. Such functional subpopulations would express glycoproteins common to many activated T cells (e.g., HLA-DR), and smaller proportions of cells would demonstrate markers for specific phases (early, intermediate, and late stages) of activation. Our findings are in keeping with this notion, and provide the first evidence, to our knowledge, of the existence of various stages of T-cell activation in inflammatory infiltrates associated with basal cell carcinomas. It is reasonable that such cells may play a role in a chronic immunologic response to a slowly evolving tumor such as basal cell carcinoma.

It is important to recognize that establishment of T-cell subtypes has little meaning if these cells do not also show evidence of ongoing functional activation. The use of monoclonal antibodies such as OKT9, OKT10, and TS2/7 should facilitate the study of activation profiles of cutaneous T-cell infiltrates in a variety of disorders. TS2/7 appears to be a marker for long-term activation of T cells. This antibody is reactive only with T cells among activated hematopoietic cells, and therefore is more specific for T-cell activation than monoclonal antibodies such as OKT9 and HLA-DR. This specificity is similar to that of the interleukin 2 (IL-2) receptor. The level of the IL-2 receptor antigen, however, has been reported to diminish contemporaneously with the expression of TS2/7 antigen [10], and the latter may therefore represent an even more sensitive probe for T cell-specific activation.

T cells expressing activation antigens were often in close proximity to tumor nests, and were associated with T6+ and HLA-DR+ dendritic cells. The epithelial cells situated at the periphery of many tumor nests showed reactivity for OKT9, suggesting that these cells may be predominantly responsible for growth and proliferation within these nests.

The precise role of activated T cells in the biologic behavior of cutaneous tumors cannot be determined from this study. In vitro assays exist, however, for the measurement of specific alloantigen-like and mitogenic stimulatory effects of normal and neoplastic cells on subsets of mononuclear cells [27]. Likewise, assays for effects of these mononuclear cells on cultured tumor cell lines have recently been described [27,28]. Use of these techniques is necessary to establish the precise nature of the interaction between activated T cells and the neoplastic cells composing cutaneous tumors.

In summary, we describe the presence of several activation antigens on T cells composing the inflammatory infiltrates of basal cell carcinomas. It is suggested that these functionally activated cells, along with similarly responsive dendritic antigen-presenting cells, may in part be responsible for the indolent clinical behavior of these tumors. Further in situ studies in immunocompromised hosts prone to the development of multiple and aggressive basal cell carcinomas, and in vitro investigations using models to assess T cell/tumor cell interactions, are indicated to further elucidate the role of local immunity in these neoplasms.

REFERENCES


