From Inflammation to Neoplasia

Mycosis Fungoides Evolves From Reactive Inflammatory Conditions (Lymphoid Infiltrates) Transforming Into Neoplastic Plaques and Tumors

THE ARTICLE by Rubegni et al[1] describes the cytokine production profile of peripheral blood mononuclear cells (PBMCs) in patients with large-plaque parapsoriasis. Interleukin 4 (IL-4) and interferon gamma (IFN-γ) were measured in PBMCs following phytohemagglutinin antigen (PHA) stimulation in patients with large-plaque parapsoriasis (LPP), patients with stage Ib (more than 10% of the body surface involved) mycosis fungoides (MF), and healthy controls. One difficulty with this approach is the bias in differentiating LPP and early patch-stage MF. As acknowledged by the authors,[1] discrimination between the 2 diseases emerges as increasingly difficult. It is not clear why 4 patients (40% in their series of patients with LPP, Nos. 4, 5, 8, and 9) whose cells exhibited T-cell receptor gamma (TCR-γ) rearrangement were included in the LPP group instead of with the early MF group. Furthermore, the question raised is how many of the patients diagnosed as having early MF had PBMCs that did not show TCR-γ rearrangement. A second controversial point concerns the controls, who should not be healthy volunteers or patients with nonneoplastic Th2-type reactions like atopic eczema. The results indicate that the cytokine pattern of LPP measured in the different categories corresponds more closely to that of normal controls than to that of subjects with MF.

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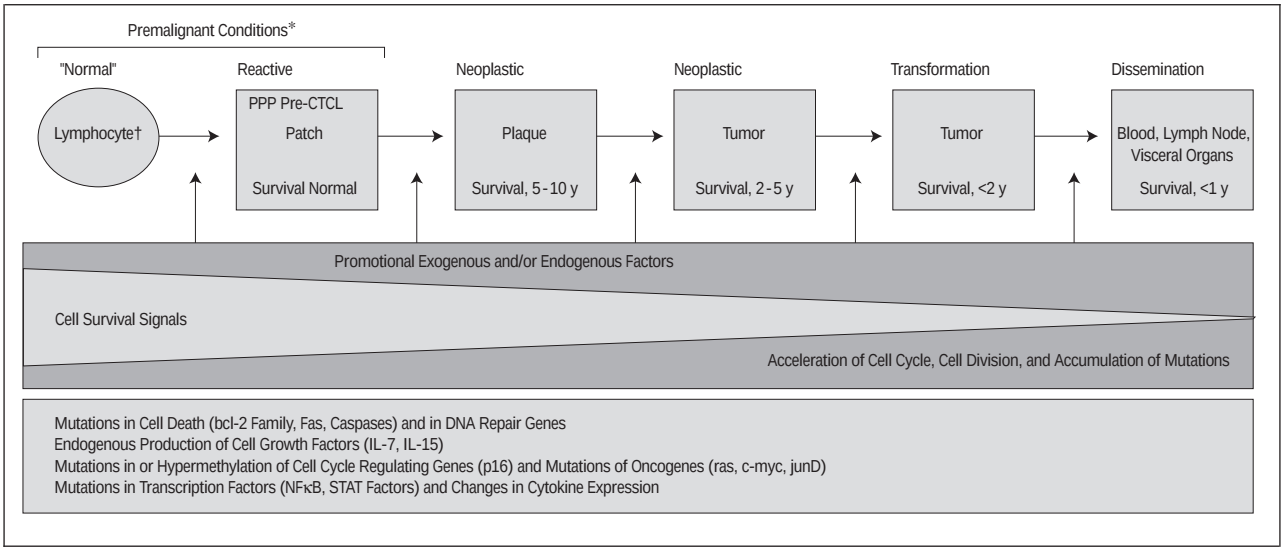
The conclusion drawn by Rubegni et al[1] from these observations is that the number of neoplastic cells may be too low or that a reactive process overcomes the neoplastic disease. However, even though referring to the case of Sézary syndrome in a young man with severe atopic dermatitis,[2] the authors do not consider the possibility of an inflammatory disorder transforming into cutaneous T-cell lymphoma (CTCL). The etiology and exact steps in the pathogenesis of CTCL are not well understood. There is increasing evidence that the formation of lymphoma and carcinogenesis are multifactorial, stepwise processes caused by the accumulation of genetic mutations, providing an explanation for the broad evolutionary and nosologic spectrum of CTCL. This pathogenetic process has been shown in colon carcinoma,[3] in human fibroblasts and epithelial cells,[4] and in human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells.[5]

With respect to the pathogenesis of CTCL, there are 2 possibilities: (1) CTCLs are neoplasic diseases from the beginning, even though definitive criteria for a neoplastic process are missing in early-stage disease; (2) preneoplastic reactive inflammatory conditions evolve into neoplasia with reproducible clinicopathologic criteria of malignancy in the transformed stages. To disprove the former statement and prove the latter, which we favor, the following null hypothesis must show a confidence limit of $P<.01$, which must be dismissed: *parapsoriasis en plaques (PPP) and preneoplastic conditions exhibit diagnostic critera of MF and do not meet criteria of reactive inflammatory processes*. If PPP and preneoplastic conditions do not exhibit criteria of MF, but of inflammatory conditions, the next question to be answered is which event or sequence of events is associated with the transition of reactive inflammatory conditions into neoplasia. By addressing this, we address the issue of the pathogenesis of CTCL. The answers to these questions are of special importance in categorizing the subtypes of lymphoproliferative disorders; the answers are also important to patients and physicians with regard to prognosis and therapy.

DEFINITIONS: INFLAMMATION VS NEOPLASIA

To discriminate between inflammation and neoplasia it is necessary to define both conditions. *Inflammation* is a reactive process, caused by irritative internal or external factors, which regresses spontaneously after cessation of the irritation. *Neoplasia*, in contrast, is a self-sustaining process with autonomous cell proliferation and the capacity for dissemination. When cell death exceeds cell proliferation, regression of tumors may occur, as seen in lymphomatoid papulosis or in keratoacanthoma.

- From a **historical** perspective, it is useful to review the original publications. With respect to MF, one should acknowledge that Alibert[6] in 1806 described a patient with plaques, ulcerated and nonulcerated tumors, but no eczematous patches. At that time—200 years ago—patients probably did not consult a physician because of faint eczematous patch skin lesions; there were more serious health problems to be dealt with.
- From a **clinical** point of view, neoplasms are locally aggressive or systemic proliferations of cells with cytogenetic relationship to the tissue of origin and a tendency to infiltrate beyond normal tissue borders and spread by metastasis.
- **Histologically and cytologically**, neoplasms are characterized by atypical morphology
- **Phenotypically**, neoplastic cells may show altered differentiation with loss of surface antigens and/or gain of tumor-associated antigens.
- **Genotypically**, clonality of proliferating cells is the hallmark of malignancy, even if not sufficient as a single



Possible pathogenetic pathway for cutaneous T-cell lymphoma. Asterisk indicates that the criteria of mycosis fungoides are not fulfilled; dagger, genomic instability; PPP, parapsoriasis en plaques; CTCL, cutaneous T-cell lymphoma; IL, interleukin; and STAT, signal transducers and activators of transcription.

criterion (ie, other criteria must be present besides clonality).

PRENEOPLASTIC CONDITIONS AND PPP DO NOT EXHIBIT DIAGNOSTIC CRITERIA OF MF

From a historical and clinical point of view, patches as seen in PPP do not fulfill criteria of lymphoid neoplasms (lymphoma) designated as MF by Alibert[6] almost 200 years ago. The cells are often surrounded by clear spaces (halos). The most important diagnostic features of of lymphoma are lymphocytes with extremely convoluted, medium-large (>7 µm in diameter) cerebriform nuclei occurring singly or clustered within the epidermis (Pautrier microabscesses) and in monomorphic sheets within the dermis. Additional significant histologic features are epidermotropism of single cells and lining up of lymphoid cells among basal keratinocytes at the dermoepidermal junction, absence of significant papillary dermal fibrosis, and absence of significant numbers of dermal blastlike cells (Santucci M, Smoller B, Biggeri A, et al, unpublished data).[7,8]

In a study performed by the International Society for Cutaneous Lymphomas,[9] the lesions clinically designated as "parapsoriasis" (n=33) showed histologic features indistinguishable from samples from the control group (n=33; eczema, psoriasis, and other inflammatory disorders) rather than from the MF group (n=33). Phenotypically and genotypically, the infiltrate of PPP usually does not show an abnormal antigenic profile or loss of differentiation antigens like CD7 and does not show clonal rearrangement of T-cell antigen receptor genes. T cells have frequently occurred in the peripheral blood but not in the skin of patients with small-plaque parapsoriasis.[9] From these data, it was hypothesized that a sufficient cutaneous antitumor response but also an extracutaneous origin of the T-cell clones might explain the failure to detect skin-infiltrating clonal T cells. In 4 of the 10 patients with PPP described by Rubegni et al[1] and observed over 14 to 36 months, clonal rearrangement of the TCR-γ was detected in skin infiltrates. One explanation for these apparently contradictionary results may be that the follow-up time was not long enough to do bias-free allocation of the cases in the different diagnostic groups. In the series of Liebmann et al,[10] 23 cases of early- or patch-stage MF diagnosed by clinicopathologic analysis of skin biopsy specimens were investigated. Of these, 18 (78%) showed TCR-γ or both β- and γ-chain gene rearrangements. In our series of patients diagnosed as having PPP at the time of first presentation (n=231) and observed over 10 to 30 years (n=18), only 1 developed clear-cut MF, according to the criteria described above, and demonstrated genotypical change from germline to clonal rearrangement of the *TCR-γ* gene.[1,10] Rubegni et al[1] claim a percentage ranging from 0% to 46% of PPP cases that progress into clear-cut lymphoma. This finding is confusing. In conclusion, there is insufficient evidence for a diagnosis of MF in PPP and premycotic conditions, which both exhibit morphologic, phenotypic, and genotypic features of reactive inflammatory processes.

THE PATHOGENESIS OF CUTANEOUS LYMPHOMAS: WHEN DOES MF START?

Mycosis fungoides starts when the criteria normally used to make a diagnosis are fulfilled. These criteria are clinical (progression to plaques or tumors), histologic and cytomorphological (atypical cells in the context of distinct histologic patterns), phenotypical (loss of differentiation markers or gain of tumor markers), and genotypical (clonal proliferation). The question to be answered is which events and/or which sequence of events on a molecular level drive lymphocytes from a reactive inflammatory premycotic disorder into a neoplastic process? One possibility is shown in the **Figure**.

There are many phenomena associated with (and proven to be unassociated with) the evolution of CTCL. However, the etiology and the exact steps in the pathogenesis of CTCL are not completely understood. Chro-

mosomal abnormalities occur regularly[11] in CTCL. An association with certain histocompatibility antigens has been described.[12] Reports on the significance of environmental factors in the pathogenesis of CTCL are contradictory. The effect of persistent antigenic stimulation by contact allergens in the pathogenesis of MF is debatable.[13,14] Molecular studies using polymerase chain reaction techniques have shown that human T-cell lymphotropic virus (HTLV-1) plays no role in CTCL other than in adult T-cell lymphoma.[15,16]

There is some controversy about the immune biology of CTCL with respect to the Th1/Th2 systems and their cytokine profiles.[17-19] The dominance of the Th2 cells[20] explains the well-known clinical phenomena seen in most patients with CTCL, such as reduced cutaneous delayed-type hypersensitivity reactions, hypereosinophilia, alterations in serum immunoglobulin levels (IgE, IgA), increased risk of second malignancies and immunological abnormalities of PBMC-like reduced natural killer cell activity, and decreased mitogen-induced proliferation.[21]

A cytokine important for the development of CTCL is IL-15, expressed by basal layer keratinocytes and skin dendritic cells.[22] Interleukin 15 interacts with the β-chain of the IL-2 receptor,[23] is a potent growth factor for the IL-2–dependent CTCL cell line SeAx, and prolongs the in vitro survival of CTCL cells isolated from patients with Sézary syndrome.[24]

In the evolution from normal to neoplastic lymphocytes, it seems that lymphocytes are driven into activation and reactive cell proliferation by an antigen that may be viral or nonviral, self-, altered self-, or cross-reactive with other antigens. They may subsequently develop genomic instability ("genotraumatic lymphocytes").[25] The risk for the occurrence of mutations in the setting of genomic instability increases with each new cell division, which is usually limited by controlling mechanisms such as programmed cell death (apoptosis). In CTCL, apoptosis is blocked by increased bcl-2 protein expression.[26]

Another mechanism by which cells normally die is cellular senescence due to excision of telomeres. These repetitive base sequences (TTAGGG) at the end of each chromosome are responsible for the maintenance of chromosomal structure and function. Immortal cells overcome this regulation by reactivation of telomerase activity. Skin-homing T-cells and PBMCs from CTCL have high telomerase activity and short telomere length. In parapsoriasis, abnormal telomerase activity characteristic of CTCL is already present.[27,28]

There have been similar findings in CTCL cell lines (unpublished data of G.B., 1998). This leads to accumulation of mutations in a stepwise sequence affecting DNA repair genes,[29] oncogenes, tumor suppressor genes, cell cycle–regulating genes,[30-34] NFκB, and signaling factors.[24,31,35-37] Finally, a highly abnormal cell clone evolves, which grows independently from external stimuli possibly due to autocrine growth-stimulating factors (eg, IL-15, IL-7, and IL-2)[21] and loss of response to growth inhibitory factors (eg, transforming growth factor β).[37] Another stimulatory factor could be the result of the interaction between costimulatory molecules B7 and CD28.[38]

CYTOGENETIC STUDIES SUPPORT THE CONCEPT OF A MULTISTEP EVOLUTION OF CTCL

Studies of bone marrow, peripheral blood, and skin tumor cells from a patient with MF at an early stage showed chromosome abnormalities in 100% of the cells harvested from the cutaneous specimen, whereas the cells of the bone marrow and blood were karyotypically normal. Three related clones occurred, showing increasing cytogenetic complexity, which suggests a polyphasic evolution of this chronic T-cell lymphoproliferative disease.[39]

Feulgen stain used with DNA cytometry allows a prognostic evaluation of CTCL.[40] Recurrent abnormalities of the genes that encode T-cell antigen receptors have not been demonstrated in CTCL.[41] The region between 1p22 and 1p36 was identified as a region of the genome that requires detailed analysis toward the identification of potential gene(s) involved in the process of malignant transformation and/or progression in MF. Unfortunately, cytogenetic studies using modern techniques have not been done to identify genetic alterations in skin lesions of premycotic conditions and parapsoriasis, probably because of the small number of dividing cells. Newer techniques of comparative genomic hybridization or fluoresent in situ hybridization may help detect early mutations.

CONCLUSIONS

We suggest that *mycosis fungoides* is a clinicopathologic term that describes a neoplasm of cerebriform T lymphocytes that form plaques and tumors. We further suggest that MF arises in a background of chronic inflammation or as a response to chronic antigen stimulation. Subsequently, a series of mutations results in the stepwise progression from eczematous patches, as seen in parapsoriasis, to plaques, tumors, and eventually hematogeneous dissemination in MF. The pathogenetic process is driven by various, probably individually different, exogenous factors (eg, environmental foreign antigens[42-44] or bacterial superantigens[45,46]) and/or endogenous factors (eg, autocrine cytokine loops and B7/CD28 interaction).

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