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Round Cells of the Epidermis: Clues from Studies on Neoplastic Lymphocytes of Cutaneous T Cell Lymphoma

RICHARD L. EDELSON, M.D.

Department of Dermatology and Cancer and General Clinical Research Centers, Columbia University, New York, New York U.S.A.

Neoplastic cells of cutaneous T cell lymphoma (CTCL) appear to be of monoclonal origin and frequently are nonspecific helpers of normal B cell differentiation. A natural progression from epidermotropic (mycosis fungoides and Sézary syndrome) to nonepidermotropic, more widely disseminated T cell neoplasms generally occurs. Affinity of CTCL cells for the epidermis may result from their having membrane receptors for histocompatibility (Ia) antigens present in skin. Cultured human epidermal cells produce a thymopoietin-like molecule, an indication of a role for skin in T cell differentiation.

Cutaneous T cell lymphoma (CTCL) is a neoplasm of thymus-derived lymphocytes (T cells) that characteristically infiltrate broad expanses of skin, frequently have functional properties characteristic of helper T cells, and may be monoclonally derived in individual patients (for a review, see reference 1). A natural progression from epidermotropic forms (mycosis fungoides and Sézary syndrome) to more disseminated and aggressive nonepidermotropic forms appears to be a general feature. Although the term "cutaneous T cell lymphoma" was proposed in 1975 [2] as a clinically and scientifically useful way of unifying the otherwise splintered group of T cell lymphomas of the skin, it was not until the 1978 National Cancer Institute Symposium that it was formally adopted and began to receive broad acceptance [3].

This paper reviews recent studies on the functional and membrane properties of CTCL cells. As neoplastic amplifications of T cells with an affinity for epidermis, these cells present special opportunities for the investigation of epidermal-T cell interactions.

HELPER FUNCTION

The first clue that CTCL cells might facilitate or "help" B cell differentiation (Fig 1) came from a preliminary observation made in collaboration with Broder and Waldmann [4]. Leukemic T cells from one patient had the property of reversing the inability of B cells from a patient with hypogammaglobulinemia to develop into immunoglobulin-secreting cells after stimulation *in vitro* with pokeweed mitogen (Fig 2). The hypogammaglobulinemic patient also had a profound thymic deficiency, manifested by the absence of delayed hypersensitivity on skin testing with specific antigens, decreased *in vitro* response to mitogens and antigens, failure to reject grafted allogeneic skin, and few peripheral blood lymphocytes with T cell membrane properties; however, despite the hypogammaglobulinemia, this patient had normal peripheral blood B cell levels. The most likely explanation was that the leukemic T cells from the CTCL patient permitted B cells from the hypogammaglobulinemic patient to differentiate into immunoglobulin-secreting cells by providing a previously absent cellular function.

Additional studies on cells from other CTCL patients were then performed. Immunoabsorbent-column-purified B cells, further depleted of T cells by having been rosetted with sheep erythrocytes, did not secrete significant quantities of IgG, IgM, or IgA, as determined by double radioimmunoassay after stimulation with pokeweed mitogen, unless T cell "help" was available. Homogeneous cell populations from 4 of 5 leukemic CTCL patients provided this polyclonal "help," and the possibility that this reflected contamination by a small number of residual normal T cells was excluded by dilution studies.

The absence of suppressor T cells in the same cell preparations was suggested by 2 observations. First, when normal purified T cell preparations were added at 4 times the optimal T-to-B cell ratio for production of maximal immunoglobulin synthesis, significant inhibition indicative of suppressor activity was identified. Second, not only was such suppressor activity absent even with increasing concentrations of leukemic T cells from our patients, but the addition of leukemic T cells as 3rd-party cells overcame the apparent suppressive effect of increasing concentrations of normal human T cells. We concluded that 4 of our 5 CTCL patients had leukemia of T lymphocytes with helper T cell activity and suggested that these neoplasms might have developed from normal helper T lymphocytes. We have recently extended these observations to include the aleukemic phases of CTCL [5]. Furthermore, these findings support our impressions that the various phases of CTCL are all manifestations of the same basic underlying process. Lawrence et al [6] also reported that T cells from a patient with CTCL had polyclonal helper activity, and Kermani-Arab et al [7] found that cells from another patient lacked concanavalin-A-inducible suppression for mitogen-responsive normal cells.

The importance of these observations may be 2-fold. First, patients with CTCL, leukemic phase, often have normal or increased levels of serum immunoglobulin despite often dramatically decreased absolute B cell levels. In addition, patients with mycosis fungoides often have elevated IgG, IgE, and IgA serum levels [8], and most of our patients with CTCL, leukemic phase, have elevated levels of IgE and IgA. Production of IgE and IgA appears to be particularly T-cell-dependent [9]. The 2nd reason for the significance of the above experimental observation concerns the nature of the cell from which such leukemias develop. Since individual lymphoproliferative disorders may represent neoplasms of lymphocytes derived from normal lymphocytes, it is possible that the cutaneous T cell neoplasms develop from normal precursors that have a tendency to migrate to the skin and that subserve the above-

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Reprint requests to: Richard L. Edelson, M.D., Associate Professor, Department of Dermatology, Columbia University, 630 West 168th Street, New York, NY 10032.

Abbreviations:
CLL: chronic lymphocytic leukemia
con A: concanavalin A
CTCL: cutaneous T cell lymphoma
EAC: sheep erythrocyte coated with Igm antibody and complement
HTLA sera: rabbit anti-human-normal-thymocyte sera
JRA: juvenile rheumatoid arthritis
LCs: Langerhans cells
MHC: major histocompatibility complex
MLC: mixed leukocyte culture
α-MM: α-methylmannoside
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis

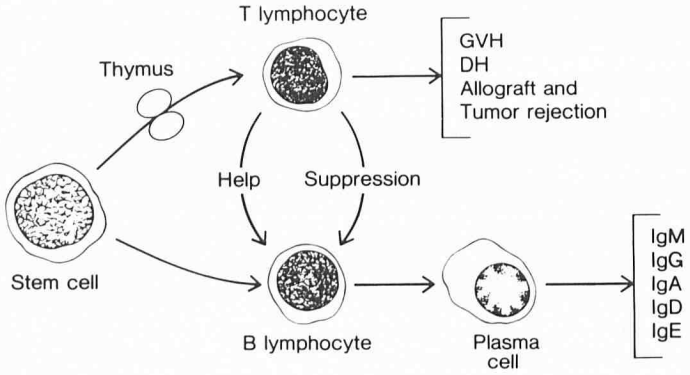

FIG 1. Lymphocyte differentiation. T cells, differentiating under the influence of the thymus, mediate graft-versus-host (*GVH*) disease, delayed hypersensitivity (*DH*), and allograft and tumor rejection. B cells are the precursors of the immunoglobulin-producing plasma cells. Distinct populations of "helper" T cells facilitate, and "suppressor" T cells suppress, B cell differentiation.

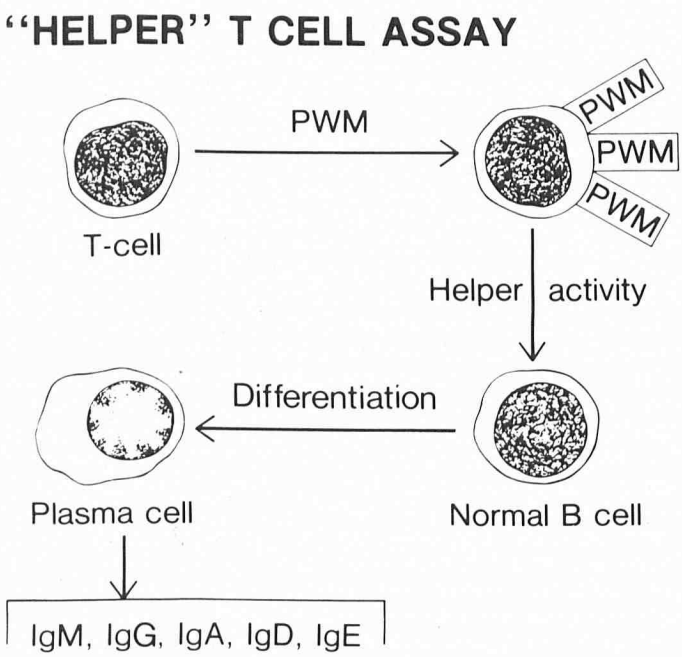

FIG 2. "Helper" T cell assay. T cells, stimulated with pokeweed mitogen (*PWM*), induce B cells with which they are cocultured to differentiate into immunoglobulin-producing cells. One can identify production of immunoglobulin directly by cytoplasmically staining cytocentrifuged cells with fluorescein-labeled antihuman Ig or by using a radioimmunoassay of cell supernatant.

described "helper" function. It is also possible that these abnormal T cells have a nonspecific helper role that is not a property of their normal ancestors.

MEMBRANE DIFFERENTIATION ANTIGENS

The above observations must be viewed in the context of information generated from studies, on experimental animals, that demonstrate a correlation between T cell functional capabilities and surface phenotypes. The conceptual framework upon which our present approach is based is that observations made in rodent systems can be extended to the investigation of human lymphocytes.

Such studies have demonstrated that suppressor T cells can specifically [10–12] and nonspecifically [13] inhibit B cell production of Ig. Similarly, specific and nonspecific helper T cells [14] have been identified. Although the specific helper T cells operate in a genetically restricted manner [15], the nonspecific ones (as with our polyclonally stimulating CTCL "helper" cells) do not [16]. Specific suppressor factors have been extracted from T cells [17]. Extracts of antigen-primed mouse thymocytes contain both suppressive and enhancing T cell factors that bear antigenic determinants coded for by the I-J and I-A subregions of the major histocompatibility complex (MHC) [18], respectively. Nonspecific suppressor T cells may predominate in spleens of young mice, and more specific suppressor T cells may predominate in the spleens of older animals [19].

Definitive evidence that individual functions are performed by distinct subclasses of T lymphocytes has been obtained through the use of specific antisera against murine Ly surface antigenic determinants [20–24]. Cells exhibiting cytotoxic and suppressor activity appear to express $Ly_{2,3}$ surface antigens, and T cells mediating delayed hypersensitivity, mixed leukocyte culture (MLC), and helper activity express the Ly_1 phenotype. Immunoregulatory circuits have been identified among subpopulations of T cells, through the use of these specific antisera [25]. Although antigen-stimulated Ly_1 cells themselves mediate helper activity, small numbers of Ly_1-positive T cells induce nonimmune other T cells to exhibit potent suppressor activity. Similarly, small numbers of Ly_1-positive T cells can apparently induce separate $Ly_{2,3}$-positive cells to exert potent, inhibitory effects in the *in vitro* generation of alloreactive cytotoxic activity [26]. In addition, $Ly_{2,3}$-positive, and perhaps $Ly_{1,2,3}$-positive, cells interact with Ly_1-positive cells in such a way that specific cell-mediated cytotoxicity is amplified [27]. These phenomena suggest that Ly_1-positive T cells may be programmed to signal other sets of T cells to fulfill their respective genetically programmed capabilities.

Other evidence that commonly studied *in vitro* systems involve complicated interactions between phenotypically distinct T cell populations has been presented by several laboratories. For example, alloantigen-activated murine spleen cells release a soluble suppressor factor [28] for which activated responding T cells have a receptor [29]. This specific activation of suppressor T cells causes nonspecific suppression of the response by syngeneic T cells to alloantigen [30]. Activation of both suppressor and helper T cells can also occur in response to mitogens. Polyclonal helper T cell activity occurs after pokeweed mitogen stimulation, whereas elimination of radiosensitive suppressor cells enhances the response [31]. Concanavalin A can, under appropriate experimental conditions, stimulate T cells to perform helper, killer, or suppressor functions [13]. In a murine system, purification of cell populations permits con A to activate Ly_1-positive cells to perform helper and $Ly_{2,3}$-positive cells to perform suppressor functions [32]. Therefore, the measurement of suppressor activity in con-A-stimulated unseparated T cell populations [33] actually indicates only net suppressor activity.

The situation in mice has been clarified further by the use of an additional surface antigen, referred to as Qa_1 [34]. Ly_1-positive cells can be further separated into Qa-positive and Qa-negative subpopulations [35]. The Qa-positive cells apparently mediate feedback inhibition through the $Ly_{2,3}$-positive T cells, and cooperation between Ly_1 Qa_1-positive and Ly_1-positive-Qa_1-negative cells is required for optimal formation of antibody by B lymphocytes. Stanton and Boyse [34] and Cantor et al [35] suggest that it may be the ratio of Qa_1-positive to Qa_1-negative T cells bearing receptors for specific antigen that determines whether an antibody-producing or -suppressing effect predominates. Approximately 67% of the Ly_1-positive cells are also Qa_1-positive. Furthermore, studies by Hayes and Bach [36] suggest that nonoverlapping populations of murine T lymphocytes express surface antigens coded for by distinct subregions of the H-2 system. Antigens coded for by the I-J subregion have been reported to be preferentially expressed on suppressor T cells [17,18]. Hayes and Bach have reported the expression of surface determinants coded for by the I-E region on a possibly separate subset of murine T cells, and it will be important to determine whether these include the subset responsible for helper T cell activity.

Certainly, the availability from patients with CTCL of large

homogeneous populations of cells with helper activity offers a multitude of opportunities to cellular immunologists. Extrapolation from the above-described animal studies suggests that these populations of "helper T cells" may permit production of the human equivalent to anti-Ly_1 antibody and may facilitate isolation of helper T cell products. Of major concern is the possibility that CTCL cells are neoplastic equivalents of Ly_1 Qa_1-positive cells that might induce feedback suppression of desirable T cell function.

A question that is central to efforts, such as ours, that attempt to produce antibodies reactive with subpopulations of T cells by immunization with neoplastic cells is: Do malignant T cells express membrane antigens distinctive of individual T cell subclasses? Again, an apparent answer has been provided by studies in a murine system. Recently Mathieson et al [37] examined several mouse lymphocytic tumors for the expression of those antigens recognized to be present in the cell membrane of normal mouse T lymphocytes. In addition to identifying Thy_1 and TL antigens on the surfaces of neoplastic cells from thymic lymphocyte tumors, these investigators also identified restricted expression of Ly phenotypes. Most of the thymic tumors expressed either Ly_1 or Ly_2 antigen, and the expression of these Ly antigens were stable over several transplant generations, an indication that they were an intrinsic property of the individual neoplasm. In addition, Ly_1 and Ly_2 were not identified on non-T-cell tumors. These findings underscore the suggestion that neoplastic T cells of CTCL can be expected to continue to express the human equivalent of Ly_1, and antigenic marker of helper T lymphocytes, if they are in fact derived from helper T cells.

In the context of these observations, several recent studies involving human normal and abnormal T lymphocytes are of major interest. Evans et al [38] produced a heterologous antiserum against purified human normal peripheral blood T lymphocytes and absorbed this antiserum with autologous B lymphoblasts to remove the non-T-cell activity. This antiserum displayed activity preferentially directed against the T lymphocytes responding in MLCs and producing lymphocyte mitogenic factor capable of nonspecifically "helping" B cells differentiate into antibody-forming cells. T cells that reacted more weakly or not at all with this antiserum included those capable of responding *in vitro* to soluble antigens or suppressing immunoglobulin production by normal B lymphocytes. Because of the analogy between the reactive cells with this particular antiserum and those mediating helper T cell activity in the murine system, Evans et al [39] referred to the population bearing antigenic determinants identified by that antiserum as "T_{H1}" cells. Reciprocal findings were identified by means of naturally occurring antibodies present in serum samples from selected patients with juvenile rheumatoid arthritis (JRA). Briefly, those T cells preferentially reacting with JRA naturally occurring antibodies included cells mediating nonspecific suppression of B cell differentiation or the response to soluble antigens, but not those mediating nonspecific enhancement of B cell differentiation or the response in MLCs. Notably, the anti-"T_{H1}" serum reacted with 50 to 60% of peripheral blood T cells and 90% of thymocytes. Subsequently, Evans et al [39] reported production of an additional anti-T-cell serum. Because further *in vitro* studies indicated that the anti-"T_{H1}" did not clearly identify nonspecific helper T cells, as had been initially thought, an antiserum was produced against the same purified human T cells and then this antiserum was absorbed not only with autologous B lymphocytes but also with human leukemic T lymphocytes. The resulting anti-T-cell serum reacted in a bimodal fashion, as determined by experiments with a fluorescence-activated cell sorter. Studies identifying "T_{H1}" involved complement-mediated lysis. Cells that fluoresced brightly constituted approximately 30 to 40% of the peripheral blood lymphocytes and contained the majority of killer activity (as determined in cell-mediated lympholysis reactions) and had decreased activity in MLCs and to most soluble antigens. The more weakly fluorescing cells, as studied after cell sorting, responded well in MLCs and to soluble antigens, and amplified the cytotoxicity manifested by the other population. Results directly reflecting helper or suppressor T cell activity for B cell differentiation were not reported. In summary, highly absorbed heterologous Ig produced against a single purified population of normal human T cells appears to have specificity for more than 1 subset of T cells. These studies emphasize the difficulty of producing monospecific heteroantisera.

In perhaps more definitive studies, antibodies were produced against purified surface antigens on human lymphoblastoid B lymphocytes and these may have had reactivity specifically for glycoprotein antigens similar to murine Ia antigens [40,41]. Schlossman et al [40] found that their "anti-P23-30" antibodies were primarily reactive with human B lymphocytes, approximately 15 to 20% of human null cells, neoplastic human B lymphocytes, and cells from the majority of patients with acute lymphatic leukemia and myelogenous leukemia. Recently, Fu et al [42] reported that a small percentage of normal circulating human T cells, cells from selected patients with T cell leukemia, and T cells grown in long-term culture were reactive with "anti-Ia" Ig. They also noted that the "Ia-bearing" T cell population apparently contained cells responsible for the generation of helper activity during MLC reactions. Clearly, it will be important to determine which of these various antisubpopulation antisera react with cells from individual patients with CTCL and whether other specific antisera can be produced with whole cells or isolated membrane components from such patients.

MEMBRANE ANTIGENS OF THE NEOPLASTIC T CELLS OF CUTANEOUS T CELL LYMPHOMA

Three distinct approaches have been taken in the investigation of antigenicity of CTCL neoplastic cells. First, circulating anti-T-cell antibodies from patients with systemic lupus erythematosus were used as probes. Second, Con A binding proteins in the cytoplasmic membranes of the cells were precipitated and studied by means of specific antisera. Third, heterologous antisera were produced by immunization with these neoplastic cells and subsequent absorption to increase the specificity of the resulting antibodies.

The study with lupus antibodies [43] was possible because of enormous numbers of cells obtained through therapeutic leukapheresis. Over a 35-day period, 36×10^{11} peripheral blood leukemic T lymphocytes were removed by leukapheresis from an otherwise untreated patient with CTCL. The recovered leukemic T cells, which were identified by their deeply indented nuclei, constituted at least 95% of the patient's circulating mononuclear leukocytes and had the T cell membrane properties of binding sheep erythrocytes and susceptibility to lysis by specific anti-human-T-cell serum and complement. These cells lacked the capacity to proliferate in response to T cell mitogens, soluble microbial antigens, or allogenic leukocytes. They also failed to stimulate normal allogeneic lymphocytes to proliferate in MLC, apparently because of a lack of the necessary membrane antigens rather than the production of inhibitory material since they failed to inhibit as 3rd parties to an otherwise normal MLC. Ohter characteristics of these lymphocytes included the ability to enhance B cell polyclonal immunoglobulin production in response to pokeweed mitogen and the *in vitro* production of a substance (or substances) that inhibited macrophage migration. B cell lymphoblasts were harvested from continuous cell cultures of human peripheral blood lymphocytes from a single normal donor. These B cells had receptors for C3 and readily detectable surface immunoglobulin; they were lysed by rabbit anti-human-B-cell serum, but were not lysed by rabbit anti-T-cell serum and did not form rosettes with sheep erythrocytes.

Briefly, immunoabsorbent columns were produced as follows. Membrane fragments from hypotonically lysed human leukemic T cells or cultured human B lymphoblasts were conjugated to CNBr-activated Sephadex G-100 columns. Antibody

applied to these columns passed through them at a rate related to the molecular weight of the immunoglobulin unless the immunoglobulin specifically bound to the attached membrane fragments. The adherent antibodies were eluted from the columns by application of glycine-HCl buffer (pH 2.5). Preparation of each column required 1.5×10^8 cells/ml of Sephadex G-100; a total of approximately 225×10^9 cells were used to form each immunoabsorbent column. Unfractionated, fractionated, and eluted antibodies were tested for inhibitory activity in MLCs and microbial antigen-stimulated cultures of normal peripheral blood mononuclear cells. Both systemic lupus erythematosus plasmas had been selected for study because they contained IgG that inhibited both sets of reactions. The results of the study indicated that both plasmas contained IgG that bound to and could be eluted from the T cell column. The anti-T, but not anti-B, antibodies significantly inhibited normal lymphocyte proliferative responses to microbial and alloantigens. These findings suggest that neoplastic T cells from the CTCL patient expressed membrane antigens that were closely associated in the normal T cell membrane with receptors for soluble and alloantigens.

In the 2nd study, the Con A binding glycoproteins were isolated from leukemic cells of 7 patients with chronic lymphocytic leukemia (CLL) and from 3 patients with leukemic phase of CTCL. These con A binding glycoproteins were then compared to the Con A acceptors expressed on cultured B cell lines and on normal thymus-dependent T cells. Cell proteins were radiolabeled by incubation either with tritiated leucine or with ^{125}I by means soluble lactoperoxidase. To specifically isolate the con A binding proteins, the Nonidet-p-40-solubilized cell membrane proteins were applied to columns of Con A-sepharose. The specifically bound cell proteins were eluted with TKM-MP-40, containing α-methylmmannoside (α-MM), and identification of radiolabeled cell membrane protein present in the α-MM eluates was achieved by means of specific antisera (alloantiserum obtained from a multiparous Amish woman and directed against certain human-B-cell-associated alloantigens; rabbit class-specific antisera against IgD and IgM or rabbit anti-β-2-microglobulin serum). Immunoprecipitation of the complex was induced by incubation with formaldehyde-fixed *Staphylococcus aureus* Cowan 1 strain bearing protein A, which binds the Fc portion of IgG. Eluates from affinity resins and specifically immunoprecipitated proteins were then subjected to separation on discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results indicated that HL-A antigens and β-2-microglobulin were present on the leukemic T cells. Two proteins with lower molecular weights (28,000 and 32,000) were seen in the SDS-PAGE profiles of the con A binding proteins of both malignant and normal T cells. Large peaks of 33,000- and 25,000-mol wt components were observed with the B cell sources, probably representing Ia antigens, but were absent in preparations from normal and malignant T cells. Therefore, although this study did not prove that the leukemic T cells failed to express Ia antigens, it did suggest that such antigens were not expressed in a large quantity. Furthermore, the failure of the leukemic T cells to proliferate in response to Con A could not be explained by any clear difference between the type of Con A binding protein on their surfaces and that found on normal T cells in the same study.

The 3rd study, which investigated surface antigenicity of these neoplastic cells, involved the production of heteroantisera by immunization with homogeneous populations of abnormal T lymphocytes [44]. Rabbit anti-human-normal-thymocyte (HTLA) sera, anti-human-B-lymphocytoblast sera, and anti-human-brain-tissue sera were tested in parallel. Prior to use *in vitro*, all of these antisera were extensively absorbed by means of human lymphocytoblasts bearing surface immunoglobulin and receptors for C3 and together expressing HL-A antigens identified on the immunizing cells. In this study, anti-HTLA caused significantly greater chromium release in 2 of the 3 patients tested, who had mean values of 92 and 89% as compared to 63.2% for the normal controls. Although anti-B-lymphoblast sera in large concentrations caused similar cytotoxicity with all cells tested, when diluted 1:128 they caused significantly greater chromium release by normal lymphocytes (mean 29.6%) than by CTCL cells (4 to 7%). Anti-CTCL-cell antiserum was less specific than anti-HTLA since it induced less chromium release from the CTCL cells, and in an associated study significantly decreased rosette formation with IgM-antibody-and-complement-coated sheep erythrocytes (IgM EACs). Together these data suggest that in addition to expressing T cell differentiation antigens, abnormal cells from some patients with CTCL may express antigens not found on the absorbing or immunizing B cell lymphoblast lines but expressed on normal circulating B cells. These findings also highlight the difficulties frequently associated with the production of heteroantisera in that activity is produced against several poorly understood membrane components.

CLONAL ORIGIN OF CUTANEOUS T CELL LYMPHOMA

Previous efforts to prove monoclonality of CTCL were unsuccessful. Probably because of both longstanding clinical disease and prior treatments with chemotherapeutic agents known to induce additional chromosome abnormalities, karyotypic analysis of CTCL cells yielded inconclusive results. The earlier studies also failed to use chromosome-banding techniques that permit identification of individual chromosomes rather than groups of chromosomes of similar size and centromere location.

We have recently completed a study that was possible because of special opportunities provided by 3 previously untreated patients [45]. In addition to widespread cutaneous infiltration, 2 of the 3 patients had at least 2 distinct body regions from which large numbers of neoplastic cells could be recovered. The 3rd patient was leukemic and had extensive bone marrow involvement. Doses of mitogens that had been predetermined to optimally induce mitosis in these cell populations were used to stimulate metaphase. Direct preparations were chromosome-banded with quinacrine staining and examined with ultraviolet microscopy. Direct observation was achieved with a trypsin-Giemsa technique. Remarkably consistent chromosome structural changes and karyotypes were identified in abnormal cells from each individual patient, an indication of the monoclonality of the malignancy. However, no single aberration was characteristic of the 3 individuals as a group. These observations, which suggest that CTCL is, in at least certain instances, a monoclonal neoplasm, are not consistent with the suggested staging system of CTCL, which is based on the concept that the process begins multifocally in skin. Cutaneous T cell lymphoma appears to be derived in a stepwise fashion from cells that normally have a propensity to infiltrate skin. Progressively more malignant subclones evolve and ultimately can be characterized by chromosome breaks and rearrangements. An individual and highly malignant subclone ultimately overgrows the original populations and causes widespread, frequently fatal, disease.

PATTERNS OF CELL PROLIFERATION IN CELL MIGRATION IN CUTANEOUS T CELL LYMPHOMA

In view of the possibility that CTCL is a monoclonal process in which widespread dissemination occurs hematogenously, it is important to discover where the cells preferentially proliferate and to which tissues the newly formed cells migrate. We studied the patterns of cell proliferation and migration in 2 patients with the leukemic phase of CTCL by obtaining serial skin biopsy samples and peripheral blood samples after intravenous administration of a pulse dose of tritiated thymidine [46]. At each sample time point, grain counts were performed on mitotic and interphase basal epidermal cells, on mitotic and interphase cutaneous neoplastic T cells, on circulating interphase leukemic T cells, and on circulating neutrophilic granu-

locytes. Average cell cycle times in these various cell populations were estimated from mean grain count halving times by a least-squares fit with an M lab computer modeling program. Cell labeling patterns indicated that the primary site of neoplastic T cell production was extracutaneous, with secondary migration into the blood and then to the skin. Rates of neoplastic cell replication were markedly greater in the extracutaneous sites. The data also indicated that the leukemic T cells appeared in the blood rapidly, in contrast to the neutrophilic granulocytes. Proliferating granulocyte precursors are known to undergo several maturational divisions in the bone marrow before being released into the blood. It appears that the leukemic T cells that were proliferating at their primary site of production were under no such constraints and were able to migrate into the blood at random. Since the circulating leukemic T cell counts did not change appreciably over the course of the study in these 2 patients, cell influx into the peripheral blood must have been balanced by cell outflow from this compartment. Therefore, rapid changes in the labeling index and particularly in the cell labeling intensity must reflect high rates of leukemic T cell migration through the various tissue compartments.

These observations scientifically substantiate 3 of our above-described impressions. First, the neoplastic T cells sequestered in skin appear to belong to a slowly proliferating cell population. Second, rapid rates of cell renewal occur in extracutaneous sites. Third, the neoplastic T cells appear to move rapidly into and out of the blood compartment.

On the basis of the results of autoradiographic studies being prepared for publication, we think the peripheral lymph node is the major candidate for the primary site of cell renewal in advanced stages of disease. Dermatopathic lymph nodes from patients eventually shown not to have CTCL had spontaneously labeled cells consistently constituting less than 2% of the total population; in contrast, histologically involved lymph nodes from patients with cutaneous T cell lymphoma had labeling indices consistently in excess of 5% and frequently as high as 10 to 12% of the total cells.

EPIDERMAL-T CELL INTERACTIONS

It will be important to determine whether what we clinically recognize as CTCL is a neoplastic amplification of an underlying skin-T cell interaction. Because the abnormal cells of this disorder express membrane properties commonly attributed to T cells, it is possible that the "epidermotropism" they exhibit by forming their characteristic intraepidermal clusters (Pautrier's microabscesses) may also be a phenomenon exhibited by their presumed normal ancestral T cells. Therefore, identification of the mechanisms underlying this T-cell-epidermal cell interaction not only may further our understanding of the pathogenesis of CTCL, but also may elucidate selected aspects of basic normal T cell biology. Certainly, one of the forces motivating cellular immunologists to investigate the cells of a wide variety of lymphoreticular malignant tumors is that information obtained from the study of these large homogeneous populations of abnormal cells presumably at fixed levels of cellular differentiation will be conducive to a better understanding of their normal counterparts [47].

Several possible explanations for this epidermal-T cell interaction have already been proposed [48]: the techniques needed to test their validity are now available. The various proposed explanations can be broadly divided into 2 major groups. First, CTCL may result from the expansion of malignant clones originally derived from normal T cells reactive against either epidermal antigens or extrinsic antigens that have become localized in skin. Second, the neoplastic T cells may "home" in skin for reasons completely independent of antigen presentation i.e., it is conceivable that both the abnormal T cells and their presumed normal ancestral (helper?) T cells express membrane determinants for which cells in the skin have complementary structures, and that both the epidermal and T cells are affected by the interaction. We favor the 2nd broad explanation because it seems quite unlikely that a majority of those T cell neoplasms of adults identified to date came from the clonal expansion of cells autoreactive against differentiation antigen (or antigens) on a particular tissue (the epidermis), and it seems equally unlikely that extrinsic antigens eliciting an immune reaction from these neoplastic T cells could be so universally expressed in skin as to cause the total body involvement frequently observed. However, both sets of possible explanations merit investigation, and they may not, in fact, be mutually exclusive.

Substantial developmental and structural similarities exist between the major component of epidermis, keratinocytes, and thymic epithelial cells. Hassall's corpuscles of the mammalian thymus display an ultrastructural organization closely resembling that of keratinizing stratified squamous epidermis [49]. Specifically, the core of each corpuscle is composed of eosinophilic material arranged in layers suggestive of keratin and is surrounded by cells containing numerous cytoplasmic granules ultrastructurally indistinguishable from the keratohyalin granules of the stratum granulosum of normal epidermis [50]. The outermost cell layer of Hassall's corpuscle resembles the basal or germ layer of the epidermis. Well-developed desmosomes connected to cytoplasmic tonofilaments within the cytoplasm of the thymic epithelial cells closely resembling those of the epidermis have also been noted. Keratinocytes express membrane differentiation antigens identifiable with appropriate sera [51]. Therefore, it is of further interest that heteroantibodies, produced by immunization of rabbits and reactive with the membranes of epidermal keratinocytes, cross-react with thymic epithelial cells, in particular with those constituting Hassall's corpuscles [52]. Specific antibodies produced by immunization of rabbits with purified keratin react very strongly by immunofluorescence with epidermal keratin and with material in the center of Hassall's corpuscles [53]. Finally, "nude" mice in which thymic epithelium fails to develop derive their name from the markedly abnormal development of the epidermal derivative, hair.

These similarities between epidermal keratinocytes and thymic epithelial cells raise the possibility that cutaneous epithelium shares one functional property with thymic epithelium: an inductive influence on T cell differentiation. Certainly, skin is not a genuine "surrogate" thymus since thymectomized mice have intact skin but lack T cell function.

The hematopoietic precursor of the thymocyte (prothymocyte) is apparently already committed to further thymocyte differentiation [54] and can be induced *in vitro* to undergo further T cell differentiation by thymoepithelial cell monolayers [55–57], by fetal thymus in organ culture [58], and by thymic hormones [59–61]. If the epidermis actually has an inductive effect on T cell differentiation, it is more likely to be on "post-thymic" T cells that have already received some form of thymic influence (either by traffic through that tissue or by action of thymic hormones) and that complete their differentiation in extrathymic sites [62]. Immunoincompetent thymocytes have been shown to emigrate from the thymus [63], and such post-thymic cells can apparently be driven to differentiate further in the periphery by repeated exposure to antigen [62]. That post-thymic differentiation can be influenced in peripheral blood human lymphocytes has been demonstrated by Vogel, Incefy, and Good, who induced the development of sheep erythrocyte receptors in a population of human blood lymphocytes by exposure to human thymic extract [64]. Therefore, a possibility investigators must explore is that post-thymic T lymphocytes can be induced to undergo further differentiation through interaction with epidermal cells, through either antigen-dependent or -independent mechanisms.

Recent studies on the membrane and functional properties of Langerhans cells (LCs) have suggested that these cells, which constitute between 2 and 4% of all epidermal cells, belong to the macrophage-monocyte series. In addition to bearing mem-

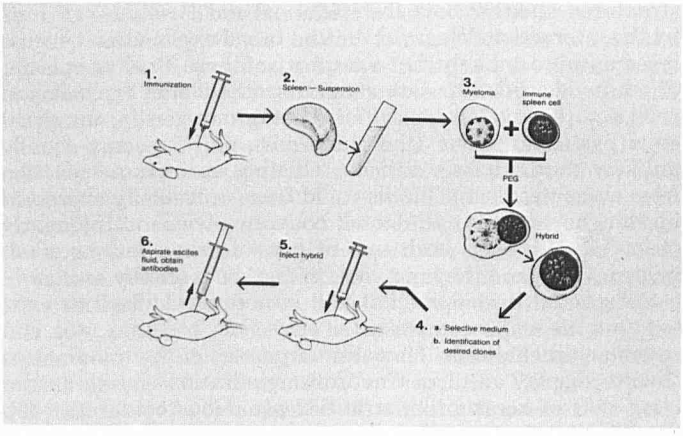

FIG 3. Production of monospecific antibody in a murine hybridoma system. After immunization of a mouse from an inbred strain with the desired antigen, a single-cell suspension of the mouse's spleen is prepared. The splenic B cells are then hybridized with multiple myeloma cells (originating from the same strain) by means of polyethylene glycol (*PEG*). The tetraploid hybridoma cells, programmed to produce unlimited amounts of Ig of the desired specificity, are isolated and then injected intraperitoneally into a mouse of the same strain. The cells proliferate and produce ascites containing high-titer monoclonally derived antibody, which can be easily harvested by peritoneal tap.

brane receptors for the Fc fragment of IgG and for C3 [65], LCs also express Ia [66]. These dendritic cells are usually found in a suprabasal position within the epidermis and have numerous widely branched, tapering processes in broad communication with keratinocytes, the major cellular component of the epidermis [67]. Involvement of LCs in contact sensitivity to allergens has been suggested because mononuclear cells accumulate around antigen-binding LCs in such reactions [68]. Guinea pig LCs stimulate allogeneic T cells effectively in mixed leukocyte reactions and are effective antigen-presenting cells [69].

These findings are of significance for several reasons. Through karyotype analysis of MLCs, we have preliminary evidence that the neoplastic cells of CTCL express receptors for Ia. The presence of cells in the epidermis that express this particular set of antigens makes it tempting to hypothesize that the T cell-skin interaction occurs, at least in part, through cell-to-cell contact involving binding of these complementary surface structures. Second, normal peripheral T cells are preprogrammed to recognize self MHC antigens even before encounter with hapten [70]. Although it is still not possible to choose between the dual recognition hypothesis [71,72], which suggests that each T cell has 2 receptors, 1 reactive with self MHC products and 1 reactive with specific antigen, or the altered-self hypothesis [73], which suggests that T cells have receptors specific for antigen-altered MHC, it seems likely that LCs can interact with either antigen-specific or uncommitted autologous T cells.

Langerhans cell induction of T cell differentiation must also be considered. Beller and Unanue [74] have demonstrated that a 40,000-mol wt factor derived from peritoneal exudate or thymic macrophages can stimulate T cell differentiation, as determined by alteration of membrane histocompatibility and differentiation antigens to more closely resemble those of mature thymocytes. Van Den Tweel and Walker [75] have demonstrated that guinea pig peritoneal macrophages can induce maturation of thymic lymphocytes to cells capable of responding to mitogens. These combined results extend those of Mosier and Pierce [76], who demonstrated macrophage induction of functional maturation of murine thymocytes *in vitro*.

An additional intriguing finding is that murine T cells and epidermal keratinocytes share tissue-specific antigenic determinants, the Thy-1 determinants [77]. Does this similarity go further? Specifically, are LCs localized in the epidermis in contact with keratinocytes, because these keratinocytes also express receptors for Ia?

CONCLUSION

Two recent scientific advances should facilitate an improved understanding of T cell-keratinocyte interaction.

First, Kohler and Milstein [78] discovered that appropriately hybridized cells can be programmed to produce enormous quantities of monospecific antibody directed against any desired immunogen. The method is summarized in Fig. 3. In essence, spleen cells from an immunized mouse are brought into suspension and are then fused, by means of polyethylene glycol, with mouse myeloma cells from the same inbred strain. These tetraploid cells retain the propensity of the myeloma cells to produce unlimited amounts of immunoglobulin, the specificity of which is now controlled by DNA from the normal spleen cells. Clones of these hybridoma cells can be screened to identify those producing antibody monospecific for T cell or epidermal cell membrane differentiation antigens. These clones are isolated and injected intraperitoneally into normal mice, where they grow as neoplasms producing very large amounts of antibody that can simply be harvested as ascites fluid. In this manner, it is now possible to obtain truly specific antihuman antibodies that can be expected to permit identification of distinctive membrane antigens on normal and neoplastic T cells, as well as receptors for complementary structures on human epidermal cells. Studies presently being completed in our laboratory have already revealed that hybridoma antibodies are extremely useful in the diagnosis of CTCL and will be reported in detail shortly.

Second, Eisenger [79] has developed a method of cultivating human keratinocytes *in vitro*, in the absence of feeder layers of fibroblasts and without addition of exogenous growth-promoting factors. These keratinocytes differentiate to a level apparently equivalent to that of cells grown in the system of Rheinwald and Green [80], and they have desmosomes and tonofilaments. We have recently demonstrated that epidermal cells grown in these cultures produce a polypeptide, which so far has been indistinguishable from thymopoietin,* the hormone secreted by thymic epithelial cells and capable of inducing early T cell differentiation [59]. These findings suggest the exceptionally exciting possibility that the epitheliotropism so characteristic of CTCL may represent a neoplastic amplification of a normal inductive influence of epidermal cells on T cell maturation.

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Announcement

Training courses in laboratory techniques including microbiology, immunology, parasitology, toxicology, and clinical chemistry are being offered throughout the year by the Bureau of Laboratories of the Center for Disease Control, Atlanta, GA. For information contact the Registrar, Bureau of Laboratories, Center for Disease Control, Atlanta, GA 30330.