The Immunohistology of Follicle Lysis in Lymph Node Biopsies From Homosexual Men

By Gary S. Wood, Carlos F. Garcia, Ronald F. Dorfman, and Roger A. Warnke

Follicle lysis is a characteristic alteration of B cell follicles described recently in lymph node biopsies from homosexual men. It consists of disruption of germinal centers by aggregates of small mature lymphocytes variably associated with erythrocyte extravasation. We studied the immunohistology of follicle lysis identified in lymph node biopsies from 11 homosexual men. The results indicate that follicle lysis has two principal immunohistologic features: (1) intrafollicular aggregates of small lymphocytes predominantly of polytypic mantle B cell phenotype (T015* /Leu-8*/μ* /λ* or λ+), and (2) disruption of the normal, unified follicular meshwork of R4/23* dendritic reticulum cells by these B cell aggregates. These structural alterations may affect the functional integrity of the germinal center as it pertains to the abnormal B cell effector function and the increased prevalence of B cell lymphoma recently documented in the acquired immunodeficiency syndrome and related disorders. Because dendritic reticulum cells weakly express the Leu-3 (T4) antigen, which is known to be an essential component of the receptor for human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) retrovirus infection, it is possible that retroviral infection of dendritic reticulum cells may play a role in the pathogenesis of follicle lysis.

RESULTS

Histopathology. Nine of the homosexual men with PGL (50%) and three of the homosexual men with AIDS (37.5%) exhibited follicle lysis involving a variable minority of germinal centers in random tissue sections (Figs 1 and 2). In one of the latter cases, follicle lysis was identified only in paraffin sections. Lymphoid follicles in the PGL cases were increased

IN A RECENT REPORT concerning the histopathology of lymphadenopathy in homosexual men, we described a characteristic alteration of germinal centers, termed “follicle lysis,” in approximately one-half of the biopsies exhibiting florid reactive follicular hyperplasia.1 This consisted of disruption of germinal centers by clusters of small mature lymphocytes with or without associated extravasation of erythrocytes. Similar features have been commented on in only a few of the reports that have appeared concerning the lymphadenopathy in homosexual men. In this study, we describe the immunohistology of follicle lysis and discuss its possible significance.

MATERIALS AND METHODS

Patients and controls. We examined lymph node biopsies from 18 homosexual men with the syndrome of persistent generalized lymphadenopathy (PGL),4 eight homosexual men with the acquired immunodeficiency syndrome (AIDS),5 and one heterosexual man with post-transfusion AIDS. Case selection was based solely on the availability of tissue accessioned in the Laboratory of Tissue Immunodiagnosis at Stanford. Controls consisted of intact follicles within biopsies from the patients and follicles within lymph node biopsies from five heterosexual patients with regional, nonspecific reactive follicular hyperplasia.

Histologic methods. All lymph nodes were obtained at surgical biopsy except for two AIDS patients, from whom tissues were obtained at autopsy within a few hours of death. Representative portions of each specimen were formalin-fixed and routinely processed into paraffin. Sections were stained with hematoxylin and eosin. Additional representative portions of each specimen were cryopreserved, sectioned, and stained as described previously. Briefly, a three-stage monoclonal antibody–biotin–avidin immunoperoxidase technique was used. The panel of monoclonal antibodies is listed in Table 1, along with their sources and specificities.

Table 1. Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antibody (Antigen)</th>
<th>Predominant Specificity</th>
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<tbody>
<tr>
<td>T015*</td>
<td>B cell lineage</td>
</tr>
<tr>
<td>Anti-mu†</td>
<td>Mu immunoglobulin heavy chain</td>
</tr>
<tr>
<td>Anti-delta‡</td>
<td>Delta immunoglobulin heavy chain</td>
</tr>
<tr>
<td>Anti-kappa‡</td>
<td>Kappa immunoglobulin light chain</td>
</tr>
<tr>
<td>Anti-lambda‡</td>
<td>Lambda immunoglobulin light chain</td>
</tr>
<tr>
<td>R4/23*</td>
<td>Follicular dendritic cells</td>
</tr>
<tr>
<td>T05*</td>
<td>C3b receptor</td>
</tr>
<tr>
<td>Mlp-P9 (Leu-M3)†</td>
<td>Monocytes, macrophages, dendritic cells</td>
</tr>
<tr>
<td>SK3 (Leu-3, T4)†</td>
<td>Helper T cells, thymocytes, histiocytes</td>
</tr>
<tr>
<td>SK7 (Leu-4, T3)</td>
<td>T cells, thymocytes</td>
</tr>
<tr>
<td>SK9 (Leu-6, T6)†</td>
<td>Thymocytes, Langerhans cells</td>
</tr>
<tr>
<td>HNK1 (Leu-7)†</td>
<td>NK cells</td>
</tr>
<tr>
<td>SK11 (Leu-B)†</td>
<td>Majority of Leu-3+ T cells, Leu-2+ T cells B cells and other leukocytes in blood and lymphoid tissue. B cells and T cells within germinal centers are virtually all Leu-8-10</td>
</tr>
<tr>
<td>9.3‡</td>
<td>Helper and cytotoxic T cells but not suppressor T cells10,11</td>
</tr>
<tr>
<td>OKT9 (T9, transferrin receptor)§</td>
<td>Thymocytes, several other proliferating cell types, including germinal center cells</td>
</tr>
<tr>
<td>OKT10 (T10)§</td>
<td>Thymocytes, plasma cells</td>
</tr>
</tbody>
</table>

Antibodies were obtained from the following sources:

*David Mason, Oxford University.
†Becton Dickinson, Mountain View, Calif.
‡Edgar Engleman, Stanford University.
§Ortho Pharmaceuticals, Raritan, N.J.
Ronald Levy, Stanford University.

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in number, enlarged, and often irregular in shape. Some exhibited attenuated mantle zones. Lymphoid follicles in the AIDS cases were smaller and, in many cases, regressively transformed (burned out) germinal centers were present. In one AIDS case, germinal center elements were totally absent.

**Immunohistology.** All cells within reactive follicles exhibited normal patterns of antigen expression as described previously. Follicular center cells (germinal center B cells) were Ia + and Leu-8 +. They weakly expressed OKT9, OKT10 and the pan-B marker T015. In contrast, mantle B cells were Ia +, OKT9 - , Leu-8 +, and strongly T015 +. They expressed both δ and μ immunoglobulin heavy chains and either κ or λ immunoglobulin light chains in a ratio of approximately 2:1.

In all cases, germinal centers exhibiting follicle lysis demonstrated disruption of the usual interwoven meshwork of R4/23 + dendritic reticulum cells (follicular dendritic cells) by aggregates of mature lymphocytes with the phenotype of polytypic mantle B cells. Admixed within these B cell aggregates were sparse T cells of either helper (Leu-3 +) or cytotoxic-suppressor (Leu-2 +) phenotype. These immunohistologic features are shown in Figs 3 through 4.

In addition, two of the nine PGL cases showed some intrafollicular aggregates of small lymphocytes that were composed almost totally of T cells. In one patient these T cell aggregates exhibited a Leu-3 + /Leu-2 + ratio >1.0, while in the other the ratio was reversed. In both patients, most of the T cells in these intrafollicular aggregates were 9.3 + and Leu-8 - . This is the normal predominant phenotype of mantle and paracortical T cells, whereas most germinal center T cells are normally 9.3 + and Leu-8 +. This suggests migration of T cells into the germinal center or abnormal expression of Leu-8 by germinal center T cells or both.

Dendritic reticulum cells in all follicles expressed their usual R4/23 +, Leu-M3 +, T05 + phenotype with weak, variable expression of Leu-3. They also stained for both κ and λ immunoglobulin light chains and μ immunoglobulin heavy chains consistent with the presence of polyclonal immune complexes.

All follicles also contained diffusely distributed minor populations of Leu-M3 + macrophages, Leu-7 + natural killer cells, and Leu-4 + T cells of both Leu-3 + and Leu-2 + subsets. All cells within follicles were Leu-6 +. The T cells diffusely admixed with follicular center cells were almost all Leu-8 - . This is typical of germinal center T cells from patients with PGL and AIDS as well as from heterosexual controls with nonspecific reactive follicular hyperplasia.

**DISCUSSION**

As previously summarized, germinal centers arise within primary B cell follicles in response to antigenic stimulation. These primary follicles are composed of lymphocytes with a mantle B cell phenotype set within a unified meshwork of R4/23 + dendritic reticulum cells. Germinal centers may subsequently disappear, undergo regressive transformation,
Fig 3. R4/23⁺ dendritic reticulum cells in follicle lysis. Frozen sections of B cell follicles in lymph node biopsies from two homosexual men with PGL show variable degrees of follicle lysis. The darkly stained R4/23⁺ dendritic reticulum cells present in much of (A) are severely disrupted into multiple discrete aggregates (arrowheads). These aggregates contain the germinal center cells apparent as cohesive cell clusters in paraffin sections such as in Figs 1 and 2. The R4/23⁺ meshwork in (B) is less severely disrupted. The unstained cells between R4/23⁺ areas in A and B (*) express the mantle B cell phenotype as shown in Fig 4. The lower portion of (A) is composed predominantly of unstained paracortical T cells (P). Immunoperoxidase stain, original magnification ×80, current magnification ×68.

or undergo progressive transformation. Regressively transformed (burned out) germinal centers consist of small nodules of R4/23⁺ dendritic reticulum cells containing blood vessels and, often, hyaline deposits. They are not specific for AIDS and have been described in various clinical settings, including angioimmunoblastic lymphadenopathy and after corticosteroid therapy. Progressive transformed germinal centers consist of large nodules of lymphocytes with a mantle B cell phenotype admixed with a variable number of clusters of typical germinal center cells. All these cells are set within a unified meshwork of R4/23⁺ dendritic reticulum cells. Progressively transformed germinal centers typically occur in the nodular lymphocyte-predominant type of Hodgkin's disease. They sometimes occur in other types of Hodgkin's disease and in nonspecific reactive follicular hyperplasia. They are unusual in AIDS and PGL but have been observed by us in one homosexual man with lymphocyte-predominant Hodgkin's disease.

Follicle lysis appears to be yet another change that germinal centers may undergo. Our results indicate that follicle lysis has two principal immunohistologic features: (1) intrafollicular aggregates of small lymphocytes predominantly of polytypic mantle B cell phenotype, and (2) disruption of the normal, unified follicular meshwork of dendritic reticulum cells by these B cell aggregates. This latter feature contrasts with the unity of the dendritic reticulum cell meshwork in regressively and progressively transformed germinal centers. These changes are most prominent in PGL, in which there is florid follicular hyperplasia, sometimes associated with mantle zone attenuation or loss. However, in this study we also observed similar changes in a few of the less hyperplastic, and at times regressively transformed, germinal centers of patients with AIDS. These findings suggest the possibility of abnormalities in B cell replication and maturation in homosexual men.

The florid reactive follicular hyperplasia seen in the PGL patients correlates with the increased median proliferative activity noted in lymph nodes in PGL by DNA/RNA flow cytometry. Evidence for aneuploid cell lines in 2/19 PGL patients studied by flow cytometry may be relevant to the increased incidence of non-Hodgkin's lymphomas in homosexual patients. Most of these neoplasms are B cell tumors and often exhibit follicular center cell differentiation, including small noncleaved, small cleaved, and large cell types.

The presence of attenuated or absent mantle zones or intrafollicular aggregates of mantle B cells in lymph nodes from some PGL and AIDS patients may reflect abnormalities in follicular center cell maturation that could conceivably predispose to the development of autonomous B cell clones. A recent report of three PGL patients with reactive follicular hyperplasia associated with monotypic surface immunoglobulin may be relevant in this regard.
Fig 4. The immunohistology of follicle lysis. Serial frozen sections of the germinal center shown in Fig 3B demonstrate that the lytic areas (*) are composed predominantly of cells that are Leu-8\(^+\) (A), δ\(^-\) (B), and strongly T015\(^+\) (C). This is the phenotype of mantle B cells. Leu-1\(^+\) T cells (D) constitute only a small minority population within lytic foci. Germinal center B cells within the nonlytic areas of the follicle exhibit their typical phenotype—weakly T015\(^+\)/Leu-8\(^+\)/Leu-1\(^+\). Immunoperoxidase stain. original magnification \(\times 80\); current magnification \(\times 68\).
all follicles in our PGL and AIDS cases exhibited polytypic immunoglobulin staining consistent with reactive polyclonal B cell activation. Such preactivation of B cells in vivo has been offered as an explanation for the decreased responsiveness of B cells from AIDS and PGL patients to T cell-dependent pokeweed mitogen and T cell-independent formalinized Staphylococcus aureus Cowan strain I (SAC) mitogen in vitro. This phenomenon has been described in Epstein-Barr virus (EBV) infection and is associated with hypergammaglobulinemia and an inverted Leu-3-leu-2 T cell ratio, such as occurs in many homosexual patients. This raises the possibility that, in addition to human T cell leukemia virus (HTLV)-infection, altered T cell subset ratios in AIDS and PGL may be related in part to EBV infection.

Within lymph nodes, a transition from floridly hyperplastic follicles to lymphocyte depletion has been described in certain monkeys suffering from an AIDS-like disease. Although it is not yet clear what proportion of PGL patients will go on to develop AIDS, this clinical transition has been documented in humans. It is uncertain whether such clinical evolution is accompanied by a histologic transition similar to that seen in the monkeys and, if so, what role follicle lysis may have in this process. Random depletion of lymph node B cells in clinically advanced AIDS is not a likely explanation, since paracortical T015 B cells are unchanged and OKT10 plasma cells are unchanged or increased relative to controls.

A large-scale study of lymph node biopsies with careful clinical correlation will be required to determine whether follicle lysis is specific for PGL and AIDS. To our knowledge, follicle lysis has not been described in other clinical settings, including viral-induced lymphadenopathy secondary to infectious mononucleosis, herpes zoster, smallpox, influenza, polio, or measles. Although EBV-induced infectious mononucleosis results in a combined paracortical and follicular pattern of lymph node hyperplasia, other viral lymphadenitis typically results in diffuse paracortical hyperplasia without prominent germinal center formation.

The dendritic cells of the B cell and T cell domains, i.e., follicular dendritic reticulum cells and paracortical interdigitating reticulum cells, respectively, provide accessory cell function important to B cell and T cell immune reactivity. Lesions of this dendritic cell network should not be overlooked as a possible etiology underling microenvironmentally specific alterations of B cell and T cell subsets. It is important to note that the presence of mantle B cells is not normally associated with disruption of the dendritic reticulum cell network. For example, both cell types coexist within the normal mantle zone and within germinal centers undergoing progressive transformation. As such, the apparent disruption of this network in the setting of follicle lysis may be a manifestation of a primary abnormality of the dendritic reticulum cells themselves.

In this context, it is of interest that HTLV-III retrovirus, which is known to infect helper T cells, is also known to infect certain types of histiocytes. The Leu-3 (T4) antigen is an essential component of the cell surface receptor necessary for HTLV-III retrovirus infection. Because this antigen is also expressed by monocytes, macrophages, and dendritic histiocytes, including dendritic reticulum cells, it will be important to determine whether HTLV-III retrovirus is capable of infecting dendritic reticulum cells in vivo. If so, this might affect their structural and functional integrity and, in turn, the structural and functional integrity of the germinal center as it pertains to the abnormal B cell effecter function and the increased prevalence of B cell neoplasia recognized in AIDS and related conditions.

ACKNOWLEDGMENT

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NOTE ADDED IN PROOF

Following the submission of this paper, our attention has been drawn to two published reports describing "destruction of the dendritic reticulum cell network" and "degenerative changes in the follicular dendritic cells," respectively. The first, by Janossy et al (An immunohistological approach to persistent lymphadenopathy and its relevance to AIDS. Clin Exp Immunol 59:1-10, 1985), suggested that this early destruction may explain the release of activated B cells into the circulation and prove to be an ominous prognostic sign, as it appears to correlate with "prodromal" symptoms. The second, by Tenner-Racz et al, described not only follicular dendritic cells showing degenerative changes, but identified retrovirus-like particles between the cytoplasmic processes of follicular dendritic cells (The Lancet, January 12, 1985, 105-106, 1985).

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