The Composite Nodule

A Structural and Functional Unit of the Reactive Human Lymph Node

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The relationship between T nodules and adjacent B-lymphoid follicles was investigated in 37 reactive lymph nodes by light microscopy and combined enzyme immunohistochemistry. In 16 cases (43%), T nodules and adjacent B-lymphoid follicles were unified in an ovoid, distinct nodular structure termed a "composite nodule." The composite nodule comprises two separate domains. The peripheral, subcapsular B domain contains all stationary and migratory elements of the B-lymphoid follicle, ie, B1$^+$ B-cells, OKT4$^+$, Leu 3a$^+$ helper/inducer T cells, HLA-DR$^+$ dendritic reticulum cells, and ANAE$^+$, AcPhase$^+$ tingible body macrophages and is surrounded by a B1$^+$, HLA-DR$^+$ lymphocytic corona displaying focal adenosine triphosphatase (ATPase) and alkaline phosphatase (AlkPhase) activity. The deep, paracortical T-domain contains all elements of the T nodule, ie, OKT4$^+$, Leu3a$^+$ helper/inducer T cells, high endothelial venules and HLA-DR$^+$, ATPase$^+$ interdigitating reticulum cells. The composite nodule is surrounded by a rim of ATPase$^+$, AlkPhase$^+$ high endothelial venules. Both domains are subject to changes in volume; thus, in follicular hyperplasia, the B domain enlarges at the cost of the T domain, and the reverse may occur in T-zone hyperplasia. Based on the striking resemblance between the composite nodule and the white pulp of the spleen, it is suggested that the composite nodule plays a major role in the triggering, helper-T-cell-dependent stimulation and subsequent maturation of antigen-responsive B cells into antibody-secreting plasma cells. (Am J Pathol 1986, 122:83-91)

IN THE CLASSIC concept of the lymph node architecture, the cortex is divided into a peripheral part containing lymphoid follicles alternating with interfollicular regions and a deep part, or paracortex.[1] In schematic diagrams, the paracortex is depicted as a diffuse, lymphocyte-rich area of rather uniform thickness underlying the entire peripheral cortex.[2] However, several morphologic findings cast doubt upon the diffuse nature of the paracortex. In rats[3] and mice[4] tridimensional studies of the deep cortex have revealed the existence of hemispherical deep cortex "units" contiguous to a portion of peripheral cortex; several such "units" may fuse to form a deep cortex "complex."[5] In man, similar deep cortex units have been found,[6] and a partial or complete nodular alteration of the paracortex is a rather common finding in a variety of reactive lymph node conditions.[7] These nodules have been termed tertiary nodules,[8] tertiary follicles,[9] and paracortical T nodules.[7,10]

We recently studied the light-microscopic features and immunohistochemical composition of paracortical T nodules in various reactive lymph node conditions.[11] T nodules were composed of a predominance of helper/inducer T lymphocytes, admixed with interdigitating reticulum cells and high endothelial venules. This cellular composition strikingly resembled the composition of dendritic cell/T cell clusters observed *in vitro*.[12] Because of the functional properties of these clusters *in vitro*[12] and the close topographical relationship of T nodules to adjacent B-lymphoid follicles, we suggested that T nodules represented the site of activation and proliferation of T cells and the place where subsequent T-cell/B-cell interactions could take place.[11]

In the present study, we have analyzed the topographical relationship between T nodules and B-lymphoid follicles in human reactive lymph nodes in greater detail. Using combined enzyme immunohistochemical techniques, we visualized a nodular struc-

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Table 1—Number, Histologic Features, and Frequency of Occurrence of Composite Nodules in Reactive Lymph Nodes, Used for the Present Study

Histological diagnosis	Number	Presence of typical composite nodules
Follicular hyperplasia*	12	1/12
Nonspecific reactive lymphadenitis†	10	7/10
Piringer's lymphadenitis‡	6	2/6
T-zone hyperplasia§	6	5/6
Polymorphous pulp hyperplasia‖	3	1/3
Total	37	16/37 (43%)

* Increased numbers of large, secondary lymphoid follicles, distributed throughout the lymph node parenchyma, with reduction of interfollicular and paracortical areas.

† Variable prominence of lymphoid follicles and paracortical area; dilatation of sinuses, filled with histiocytes; variable numbers of plasma cells in medullary cords.

‡ Hyperplastic lymphoid follicles; scattered small clusters of epithelioid histiocytes; immature sinushistiocytosis.[13]

§ Inconspicuous lymphoid follicles; expanded, nodularly transformed paracortical area rich in high endothelial venules and interdigitating reticulum cells.[14]

‖ Variable prominence of lymphoid follicles; expanded interfollicular and paracortical area, filled with large transformed and immunoblastic lymphoid elements, large numbers of plasma cells, and increased numbers of high endothelial venules.

ture in the antigen-stimulated human lymph node. This ovoid nodular structure contained the subcapsular follicle as well as a part of the underlying paracortex. We termed this structure "composite nodule." Because the composite nodule contains both the B and T microenvironment, we suggest that it represents the structural and functional unit of the human reactive lymph node.

Materials and Methods

Thirty-seven lymph nodes displaying a variety of reactive changes were used for the present study. Their histologic features are listed in Table 1. Thirty-four specimens were of peripheral origin (12 axillary, 19 cervical, 3 inguinal), and 3 specimens were of mesenteric origin. The patients from whom these lymph nodes were obtained (15 male, 19 female) varied in age, ranging from 9 to 67 years (median, 43 years). All lymph nodes were received fresh. Representative parts of each specimen were quickly frozen in liquid nitrogen-cooled isopentane, stored at −75 C, and used for immunohistochemistry and enzyme histochemistry. The remaining parts of each specimen were fixed in Bouin's and B5 fixative, embedded in paraffin, and used for routine light-microscopic examination.

The topographical relationship between T nodules and B-lymphoid follicles was assessed with the aid of a combined enzyme-immunohistochemical technique. In the first stage of this staining procedure, cell surface markers were demonstrated with the use of an indirect immunoperoxidase procedure. Five-micron cryostat sections were dried, fixed for 10 minutes in acetone at 4 C, and briefly rinsed in phosphate-buffered saline (PBS), pH 7.4. The following monoclonal antibodies were applied for 30 minutes at room temperature: OKT4[15] and Leu 3a[16] detecting helper/inducer T lymphocytes; OKT8[17] detecting supressor/cytotoxic T lymphocytes; B1[18] reacting with all mature B lymphocytes; and anti-HLA-DR[19] reacting with HLA-DR or Ia-like antigens. Helper/inducer T cells were visualized by a simultaneous application of both OKT4 and Leu 3a, as reported previously.[20] Monoclonal antibodies OKT4 and OKT8 were purchased from Ortho Pharmaceutical Corp. (Raritan, NJ); Leu 3a and anti-HLA-DR were obtained from Becton-Dickinson (Sunnyvale, Calif); and B1 was purchased from Coulter Clone (Hialeah, Fla). Subsequently, sections were washed for 15 minutes in three changes of PBS, pH 7.4, and treated for 30 minutes with peroxidase-conjugated rabbit anti-mouse Ig (DAKO, Copenhagen, Denmark), diluted 1:30 and containing 10% normal human serum. The reaction product was visualized with the use of 3-amino-9-ethylcarbazole and H_2O_2,[21] resulting in bright-red–stained immunoreactive sites. In selected cases (see below), the reaction product was developed with the use of a metallic ion ($CoCl_2$) solution of 3,3′-diaminobenzidine,[22] resulting in grey-black staining of immunoreactive sites.

In the second step of the staining procedure, adenosine triphosphatase (ATPase), alkaline phosphatase (AlkPhase), acid phosphatase (AcPhase) and α-naphthyl acetate esterase (ANAE) activities were demonstrated by classic enzyme-histochemical techniques[23] on the slides previously immunostained with either monoclonal antibody. ATPase and AcPhase were demonstrated with a lead salt method[23] and AlkPhase with a cobalt salt method[23] resulting in black staining of enzyme-active sites; ANAE was demonstrated by a diazonium salt technique using hexazonium-*p*-rosaniline as substrate,[23] resulting in red staining of enzyme-active sites. In order to discriminate ANAE positivity from red-stained immunoreactivity, the reaction product of the immunostaining was developed with a metallic ion solution of 3,3′-diaminobenzidine.[22]

Controls, which were invariably negative, consisted of application of nonimmune mouse ascites (Cappel Laboratories, Cochranville, Pa) or omission of secondary, peroxidase-conjugated antibodies for evaluation of endogenous peroxidase activity.

Results

Light-Microscopic Findings

In 19 of 37 lymph nodes (51.3%) T nodules were observed; their histologic features have been described pre-

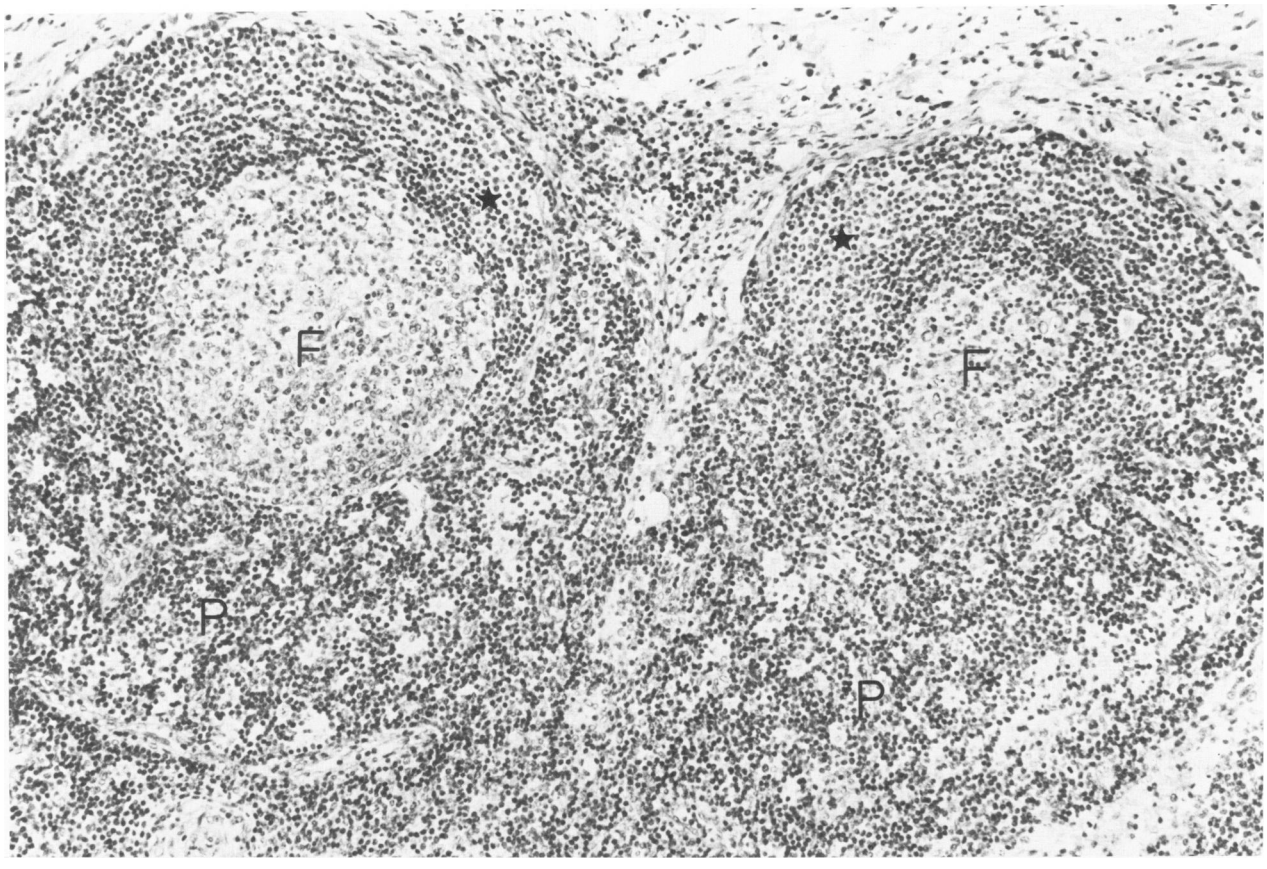

Figure 1—Two composite nodules in a case of nonspecific reactive lymphadenitis. Each composite nodule contains an upper, follicular (*F*) part and a lower, crescent-shaped paracortical (*P*) part. The lymphocytic corona is thinned at the lower pole of the lymphoid follicle; at the upper pole, a marginal zone partly envelopes the corona (*asterisk*). The starry-sky pattern in the paracortical part is due to scattered interdigitating reticulum cells. Both composite nodules are surrounded by high endothelial venules and separated from each other by an intermediary sinus. (H&E, ×160)

viously.[11] Six cases contained primary and secondary T nodules; the other 13 cases demonstrated secondary T nodules only. As reported previously,[11] secondary T nodules were frequently found to border upon adjacent lymphoid follicles; in 16 cases (43%), the secondary T nodule and adjacent lymphoid follicle were unified in a distinct large, ovoid-shaped nodular structure which extended from the marginal sinus into the paracortex. Such nodular structures will further be referred to as "composite nodules."

The occurrence of composite nodules in the various types of reactive lymphadenitis is given in Table 1. Composite nodules were most frequently observed in cases showing nonspecific reactive lymphadenitis (7/10) and T-zone hyperplasia (5/6) but were rarely found in cases showing pure follicular hyperplasia (1/12). No relationship was found between the occurrence of composite nodules, on the one hand, and the anatomic site of the lymph node or age or sex of the patient, on the other.

Composite nodules consisted of two separate domains, ie, a lymphoid follicle, situated at the upper sinusoidal pole, and a paracortical area, situated at the deep parenchymal pole (Figure 1). The lymphoid follicle demonstrated in many cases polarization of its follicular center, the dark part of it bordering upon the paracortical part of the composite nodule. The surrounding lymphocytic corona varied in thickness and was markedly reduced or even absent at the deep pole, which resulted in direct contact between the dark part of the follicular center, on the one hand, and the paracortical part of the composite nodule, on the other. At the upper pole, a marginal zone[13] was found to envelop partly the lymphocytic corona in some cases (Figure 1).

The roughly triangular or crescent-shaped paracortical part of the composite nodule showed the histologic features of a paracortical T nodule. The cellular composition of paracortical T nodules was investigated extensively in a previous study.[11] The paracortical part of the composite nodule consisted of small, round lymphocytes, interdigitating reticulum cells, high endothelial venules, and various numbers of scattered, large lymphoid cells with the cytologic features of noncleaved follicular center cells and immunoblasts.

Except at the upper pole, where the lymphoid follicle bordered directly upon the marginal sinus, the com-

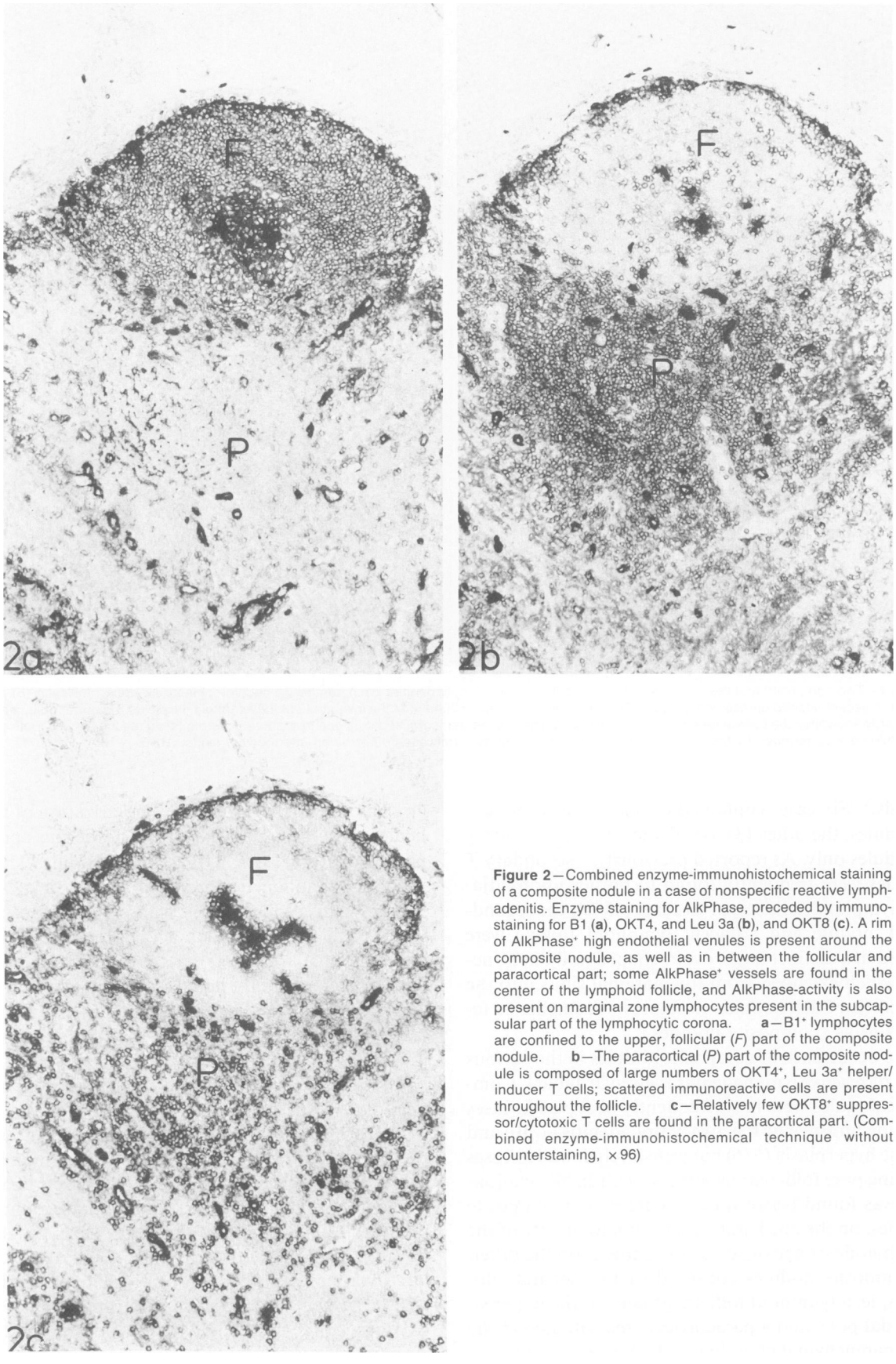

Figure 2—Combined enzyme-immunohistochemical staining of a composite nodule in a case of nonspecific reactive lymphadenitis. Enzyme staining for AlkPhase, preceded by immunostaining for B1 (**a**), OKT4, and Leu 3a (**b**), and OKT8 (**c**). A rim of AlkPhase$^+$ high endothelial venules is present around the composite nodule, as well as in between the follicular and paracortical part; some AlkPhase$^+$ vessels are found in the center of the lymphoid follicle, and AlkPhase-activity is also present on marginal zone lymphocytes present in the subcapsular part of the lymphocytic corona. **a**—B1$^+$ lymphocytes are confined to the upper, follicular (*F*) part of the composite nodule. **b**—The paracortical (*P*) part of the composite nodule is composed of large numbers of OKT4$^+$, Leu 3a$^+$ helper/inducer T cells; scattered immunoreactive cells are present throughout the follicle. **c**—Relatively few OKT8$^+$ suppressor/cytotoxic T cells are found in the paracortical part. (Combined enzyme-immunohistochemical technique without counterstaining, ×96)

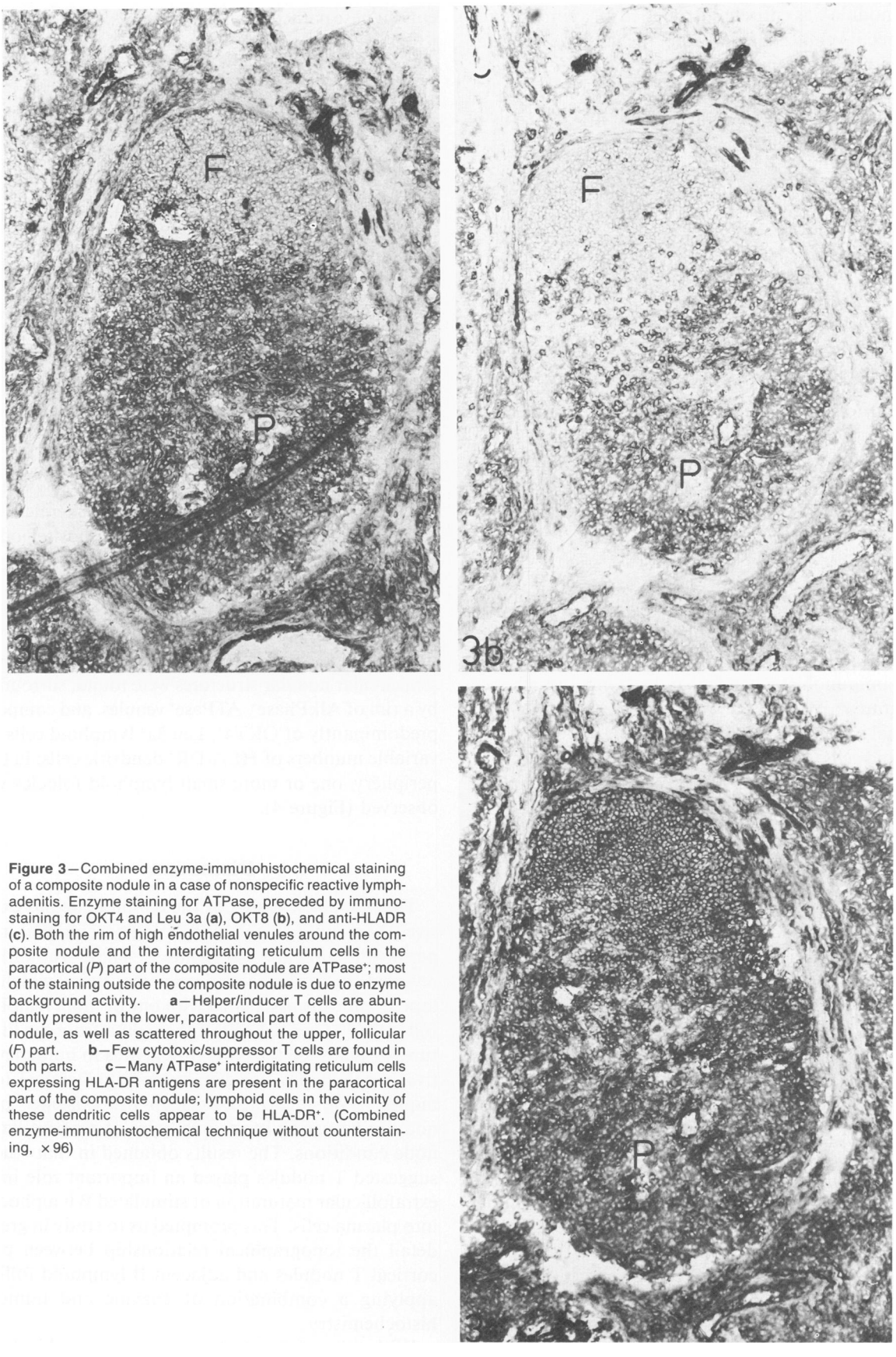

Figure 3—Combined enzyme-immunohistochemical staining of a composite nodule in a case of nonspecific reactive lymphadenitis. Enzyme staining for ATPase, preceded by immunostaining for OKT4 and Leu 3a (**a**), OKT8 (**b**), and anti-HLADR (**c**). Both the rim of high endothelial venules around the composite nodule and the interdigitating reticulum cells in the paracortical (*P*) part of the composite nodule are ATPase$^+$; most of the staining outside the composite nodule is due to enzyme background activity. **a**—Helper/inducer T cells are abundantly present in the lower, paracortical part of the composite nodule, as well as scattered throughout the upper, follicular (*F*) part. **b**—Few cytotoxic/suppressor T cells are found in both parts. **c**—Many ATPase$^+$ interdigitating reticulum cells expressing HLA-DR antigens are present in the paracortical part of the composite nodule; lymphoid cells in the vicinity of these dendritic cells appear to be HLA-DR$^+$. (Combined enzyme-immunohistochemical technique without counterstaining, ×96)

posite nodule was entirely surrounded by a rim of high endothelial venules, demarcating the nodule from the surrounding parenchyma. Singular composite nodules were separated from each other by cortical intermediary sinuses. In 10 cases, numerous plasma cells filled up the medullary cords.

In the remaining 21 cases, typical composite nodules as outlined above could not be recognized; however, morphologic variants were noted. In 12 cases of follicular hyperplasia, we recognized numerous large, reactive lymphoid follicles, bordered at one pole by a thin, small triangular rim composed of small, round lymphocytes, some high endothelial venules, and a few, if any, interdigitating reticulum cells.

In 5 of 6 cases of T-zone hyperplasia, we observed, in addition to typical composite nodules, large lymphocyte-rich semicircular nodules, surrounded by high endothelial venules. Typical interdigitating reticulum cells were situated preferentially at the outer margins of these nodules. In their periphery, underneath the marginal sinus, one or more small lymphoid follicles were observed.

Combined Enzyme-Immunohistochemical Findings

On combined enzyme immunohistochemistry, the lymphoid follicle of the composite nodule showed its classic immunohistochemical and enzyme-histochemical features[24,25] (Figure 2). The lymphocytic corona and marginal zone were composed of B1$^+$, HLA-DR$^+$ small lymphoid cells (Figure 2a), admixed with some OKT4$^+$, Leu 3a$^+$ (Figure 2b) and virtually no OKT8$^+$ lymphocytes (Figure 2c). At the upper pole, marginal-zone lymphocytes showed discrete AlkPhase activity (Figure 2). The follicular center demonstrated a meshwork pattern of reactivity upon staining with monoclonal antibodies B1 (Figure 2a) and anti-HLA-DR and contained variable numbers of OKT4$^+$, Leu 3a$^+$ (Figure 2b) and virtually no OKT8$^+$ lymphocytes (Figure 2c). Small blood vessels in the follicular center stained intensely for AlkPhase (Figure 2). Few large cells in the follicular center and many cells in the marginal sinus demonstrated ANAE and AcPhase activity, and reacted with monoclonal antibodies OKT4 and Leu 3a, consistent with their macrophage lineage.[26]

The paracortical part of the composite nodule showed the immunohistochemical and enzyme-histochemical features of a secondary paracortical T nodule[11] and was composed of an overwhelming majority of OKT4$^+$, Leu 3a$^+$ T cells (Figure 3a), few OKT8$^+$ lymphocytes (Figure 3b), and no B1$^+$ lymphocytes (Figure 2a). Large numbers of dendritic cells, as well as some of the lymphocytes throughout this area, were HLA-DR$^+$ (Figure 3c). Although it was difficult to determine whether true membrane staining was present on these paracortical lymphocytes, T cells adjacent to dendritic processes appeared to be HLA-DR$^+$, whereas T cells located away from dendritic cells were HLA-DR$^-$. The dendritic cells invariably demonstrated ATPase activity (Figure 3). Very few large cells in the paracortical area displayed ANAE or AcPhase activity.

In a few cases, a narrow rim of B1$^+$ small lymphoid cells, extending from the lymphocytic corona of the adjacent lymphoid follicle, surrounded the paracortical part to some degree.

Except at its upper, capsular pole, the composite nodule was entirely surrounded by AlkPhase$^+$, ATPase$^+$ venules (Figures 2 and 3). Variable numbers of venules with prominent endothelium were found scattered throughout the composite nodule (Figure 2); exceptionally, a rim of venules showing AlkPhase and ATPase activities separated both parts of the composite nodule from each other (Figure 2).

In specimens showing follicular hyperplasia, the small crescentic rim adjacent to hyperplastic lymphoid follicles demonstrated all immunohistochemical features of the paracortical area of the composite nodule. Even these nodules were surrounded by compressed venules. Conversely, in cases of T-zone hyperplasia, the nodules demonstrated on immunohistochemistry the presence of small B-lymphoid follicles adjacent to a hyperplastic T area. In two of these cases, very large, semicircular nodular structures were found, surrounded by a rim of AlkPhase$^+$, ATPase$^+$ venules, and composed predominantly of OKT4$^+$, Leu 3a$^+$ lymphoid cells and variable numbers of HLA-DR$^+$ dendritic cells; in their periphery, one or more small lymphoid follicles were observed (Figure 4).

Discussion

The paracortical area of the lymph node has extensively been studied with morphologic techniques in experimental animals[3-5] and man.[6,7] These studies have revealed the existence of deep cortex units, which are topographically associated with one or more lymphoid follicles.[3,5,6] On the basis of these data from the literature, we analyzed the paracortical area in human reactive lymph nodes using immunohistochemical techniques.[11] We identified as such the existence of T nodules occurring in various types of reactive lymph node conditions. The results obtained in that study[11] suggested T nodules played an important role in the extrafollicular maturation of stimulated B lymphocytes into plasma cells. This prompted us to study in greater detail the topographical relationship between paracortical T nodules and adjacent B-lymphoid follicles applying a combination of enzyme and immunohistochemistry.

With this staining technique, a topographical rela-

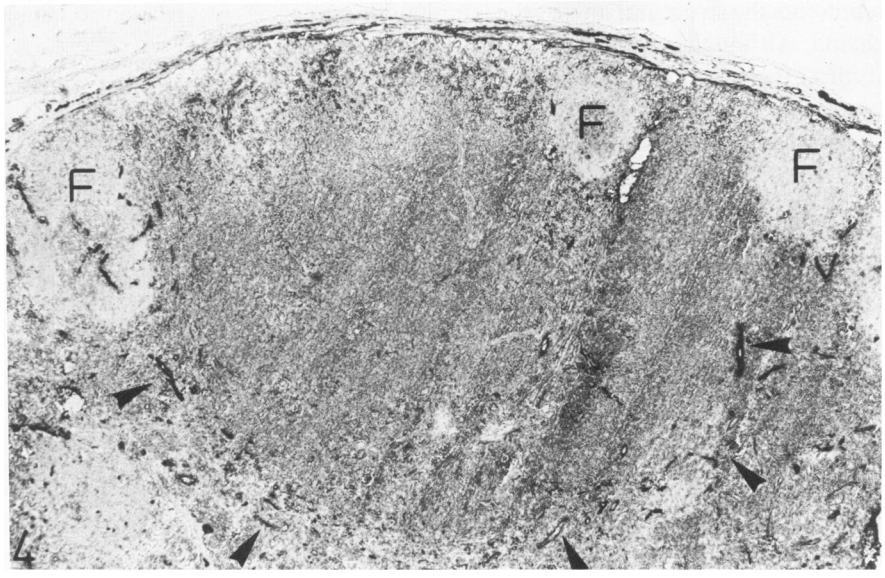

Figure 4—Combined enzyme-immunohistochemical staining in a case of T-zone hyperplasia. Enzyme staining for ATPase, preceded by immunostaining for OKT4 and Leu 3a. A large, semicircular structure, surrounded by ATPase$^+$ high endothelial venules (*arrowheads*) and composed predominantly of OKT4$^+$, Leu 3a$^+$ helper/inducer T cells, contains several lymphoid follicles (*F*) in its periphery. (Combined enzyme-immunohistochemical technique without counterstaining, ×40)

tionship was found between secondary paracortical T nodules, on the one hand, and adjacent B-lymphoid follicles on the other, which resulted in distinctive nodular structures; retrospectively, these structures could be recognized on H&E-stained sections as well.

These nodular structures, which we termed composite nodules, appeared in various morphologic forms, ranging from a large lymphoid follicle bordered by a small crescentic paracortical part to a large semicircular paracortical nodule containing one or more small lymphoid follicles in its periphery. In its most characteristic form, the composite nodule is a rounded or ovoid-shaped nodular structure composed of two separate domains. The upper part is a B domain and exhibits the classic light-microscopic, enzyme-histochemical, and immunohistochemical features of a B-lymphoid follicle.[10,24,25] The lower part is a T domain and exhibits the light-microscopic, enzyme-histochemical and immunohistochemical features of a secondary T nodule; these features were described extensively in a previous study.[11] Both domains are unified in a single nodular structure largely surrounded by a rim of venules with prominent endothelium displaying AlkPhase and ATPase activity. These morphologic and enzyme-histochemical features render these venules the characteristic of high endothelial venules.[10,14] Although high endothelial venules are classically considered to occur only in the paracortical area, previous stereographic electron-microscopic reconstruction[27] and microangiographic techniques[28] have convincingly demonstrated that well-developed high endothelial venules are present at the margin of the lymphoid follicle as well. The numerous scattered high endothelial venules associated with the T domain of the composite nodule probably correspond to the glomeruluslike tufts associated with a collection of small lymphocytes and observed in the deep cortex of the rat lymph node from the third day of the immune response onward.[28]

The morphologic, immunohistochemical, and enzyme-histochemical resemblance of the composite nodule in the reactive lymph node and the malpighian body of the white pulp in the human spleen is striking. Classically depicted as an ovoid or 8-shaped coherent structure, the malpighian body also contains well-demarcated B and T areas.[29–32] The B area, all or not displaying a follicular center, is composed of dendritic reticulum cells, B1$^+$ lymphoid cells, and variable numbers of helper/inducer T cells.[29–32] AcPhase and ANAE activities are observed in tingible body macrophages in the follicular center.[33] The T area of the malpighian body comprises the periarteriolar lymphocyte sheath and is composed of a predominance of OKT4$^+$, Leu 3a$^+$ helper/inducer T cells admixed with variable numbers of HLA-DR$^+$ interdigitating reticulum cells and a few OKT8$^+$ suppressor/cytotoxic T cells.[29–32] The malpighian body is surrounded by a marginal zone, composed of B1$^+$ marginal-zone lymphocytes[30,32] and AcPhase$^+$, ANAE$^+$ marginal-zone macrophages.[34] Similarly, variable numbers of B1$^+$, AlkPhase$^+$ cells corresponding to marginal zone lymphocytes, as well as AcPhase$^+$, ANAE$^+$ macrophages were found to surround the composite nodule to a variable extent. The latter macrophages in the mesenteric lymph node have recently been found to represent the nodal counterpart of splenic marginal-zone macrophages by virtue of their identical surface phenotypes.[35]

The malpighian body of the white pulp represents the structural and functional unit of the spleen.[36] The striking resemblance between the malpighian body and the composite nodule may indicate that the latter

represents the structural unit of the lymph node parenchyma. Although we did not perform any functional studies, the composite nodule may represent a functional unit as well. Both the malpighian body and the composite nodule are composed of closely associated B and T domains, therefore representing the ideal microenvironment where trapped antigens or antigen-antibody complexes are presented to antigen-responsive lymphocytes. B lymphocytes are triggered by antigens exposed at the surface of dendritic reticulum cells present in the B domain of the composite nodule[37] and undergo a series of transformational events in the follicular center. In the concept of Lukes and Collins,[38] the terminally transformed B cells are present in the deep or dark part of the follicular center, ie, at a site where the follicular center is in direct contact with the adjacent T domain of the composite nodule. In analogy with the *in vitro* model in which antigen-specific B lymphocytes actually enter dendritic cell/T cell clusters,[12] transformed B lymphocytes leaving the follicular center would enter the T domain of the composite nodule, where they become stimulated by helper/inducer T cells and their soluble products. Subsequently, these cells would leave the composite nodule and appear as plasma cells in the medullary cords. The absence of $B1^+$ cells in the T domain of the composite nodule is in agreement with the progressive loss of this marker during extrafollicular plasma cell transformation.[24,39]

From our results, it appears also that the composite nodule is a flexible structure whose B and T domains are subject to considerable changes in size. Thus, in follicular hyperplasia, the composite nodule consists of an enlarged B domain and a small rim of adjacent T domain; and in T-zone hyperplasia, the T domain increases in volume and may fuse with T domains of adjacent composite nodules, which results in very large nodular structures containing several small lymphoid follicles in their periphery. The reasons for this selective expansion of one or the other domain of the composite nodule are at present unclear but may be related to the nature of the challenging antigen and the stage of the immune response.

In summary, we have described the morphologic, enzyme-histochemical, and immunohistochemical characteristics of a distinct nodular structure occurring in human reactive lymph nodes. This nodular structure, termed the composite nodule, contains all elements of the B and T microenvironment and hence may represent a basic structural and functional unit of the human reactive lymph node.

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