conclude that real monocytoid B cells represent a B-cell subset unrelated to marginal zone B cells of the spleen and of the lymph nodes.

In harmony with this conclusion are our data concerning rearranged Ig genes. Monocytoid B cells in most instances (75%) harbor unmutated Ig genes, indicating their derivation from pre–germinal center B cells. In contrast, marginal zone B cells originate, in the vast majority, from mutated post–germinal center B cells as evidenced by our and others’ investigations.2,3 Tierens et al’s findings that the majority of nodal marginal zone B cells carry mutated IgH genes and are thus derived from mutated memory B cells clearly supports our conclusion that monocytoid B cells represent a different B cell subset. Furthermore, Tierens et al found a relatively high number of nonfunctional Ig rearrangements (37.5%) in their nodal marginal zone B cells.1 Because there is no subset of B cells other than germinal center B cells that carry nonfunctional IgH rearrangements at that high frequency, it is reasonable to assume that these cells most likely represent germinal center B cells. Because Tierens et al also did not provide an alternative explanation in their letter, a germinale center derivation of these nonfunctional B cells is still a matter of high probability.

Tierens et al’s discussion concerning the “clonality” of marginal zone B cells and monocytoid B cells is also unfortunately misleading. Generally speaking, proliferating cells always produce cell clones. The extension of these cell clones depends on various factors such as the number of cells in cycle, duration of proliferation, preferred (biased) mitotic division of certain cells, and so forth. We estimated the growth fraction by determining the proliferation index (Ki-67 staining) and found more monocytoid B cells in cell cycle than marginal zone B cells. In line with this observation, by single-cell analysis we found some identically rearranged monocytoid B cells, whereas marginal zone B cells were unrelated in all instances. In germinal centers many clonally related B cells have been repeatedly demonstrated by many investigations, including our own.2,4 Tierens et al performed denaturing gradient gel electrophoresis (DGGE) analysis for the determination of clonality, but we have several concerns about the reliability of their results: (1) Germinal centers are known to consistently contain huge numbers of proliferating B cells and large B-cell clones. Unexpectedly, in Tierens et al’s study they proved to be clearly polyclonal by DGGE analysis in many instances (see lanes 5, 7, 15, and 17 of their figure 5).1 (2) As stated in their paper, “clonal . . . rearrangements could also be observed when analyzing marginal zones containing only few or no cycling cells.” This indicates that DGGE can create artificial clonal patterns. (3) As stated in their letter, “small clones of B cells were demonstrated in the monocytoid B-cell and splenic marginal zones.” But upon comparison of the results provided in their paper (figures 4 and 5), the degree of clonality of the marginal zones clearly exceeded that of the germinal centers in several instances. To conclude, although the number of cells investigated is smaller in a single-cell approach, the results obtained with this technique are obviously more representative and more reliable than those obtained by microdissection and DGGE.

Finally, we are of course aware of the possibility that Ig heavy-light transcripts can occur in B cells. But these germ-line transcripts are mainly found in germinal centers prior to class-switch recombination and plasmacellular differentiation.5 Because class switching and plasmacellular differentiation have not been observed in monocytoid B cells, the meaning of Ig transcripts in these cells requires further investigation.

In summary, our data provide convincing arguments that monocytoid B cells are distinct from marginal zone B cells and represent a unique B-cell subset. It would appear most likely that differences in our results and in Tierens et al’s results are due to technical problems, analysis of different cells, and/or the selection of cases unsuitable for the investigation of monocytoid B cells.

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References

To the editor:

Ly-1b cells and Castleman disease

We read with interest and some disappointment the paper by Dupin et al on HHV-8 in Castleman disease (CD).1 Regarding their observations concerning the evolution of clonal populations of HHV-8-positive B cells in this study, Dupin et al raise questions concerning the histogenesis of these cellular proliferations and fail to recognize that these questions were previously addressed in an article we published 4 years ago. In that paper, we immunophenotypically and genotypically analyzed an unprecedented number of cases of CD (n = 63) without clinical risk factors for HIV infection and demonstrated exclusively lambda-restricted plasma cells, immunoglobulin gene rearrangements, and non-Hodgkin lymphoma.2 We also postulated that an immunophenotypically distinctive B-cell subset accumulated in the mantle zone of plasma-cell (PC) CD, which was the precursor cell of the lambda-restricted neoplasms in these patients. The KiB-3 negative phenotype of the mantle zone lymphocytes in CD was consistent with the phenotype of Ly-1b lymphocytes,3 which preferentially demonstrate lambda immunglobulin light chain rearrangement3 and express lambda immunoglobulin light chains.4 Ly-1b lymphocytes, although

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characterized as fetal (Ly-1) B lymphocytes, are found in adult patients and are produced in bone marrow like adult (Ly-2) B lymphocytes.5

Dupin et al’s finding of latent nuclear antigen expression in the scattered plasmablastic cells in the mantle zone of patients with CD suggests that HHV-8 may selectively transform a subset of Ly-1b lymphocytes that reside in mantle zones in CD and may subsequently develop interfollicular microlymphomas in HIV infected patients with CD. We speculate that, because Ly-1b cells are important in the control of immune reactions involving recognition of self and non-self antigens, they may be associated with the development of anti-myelin-glycoprotein autoantibody formation and neuropathy in CD patients with the POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal paraprotein, and skin changes) syndrome. Because interleukin-6 (IL-6) induces plasma cell differentiation from B-cell precursors, it is produced by follicular dendritic reticulum cells and scattered interfollicular cells in CD,6-8 and is responsible for systemic symptoms in CD patients,9 IL-6 may also induce plasma-cell differentiation from mantle-zone Ly-1b lymphocytic precursors in PC CD. Because only a subset of HIV-negative CD patients are infected with HHV-8,10,11 because HHV-8 negative patients develop CD associated with the POEMS syndrome, and because systemic symptoms in HIV/HHV-8–infected CD patients resolve with human IL-6 neutralizing monoclonal antibodies,12 it is likely that human IL-6 rather than viral IL-6 is the principle pathogenic cytokine in PC CD.

The importance of Ly-1b (fetal) lymphocytes in the pathogenesis of hyaline vascular CD is suggested by the concentric accumulation of Ki-B3 mantle-zone lymphocytes in hyaline vascular disease, the similarities in anatomical distribution of Ly-1b lymphocytes, the clinical distribution of hyaline vascular CD,13-16 and the reported series of CD in children.17 Similarities in pathogenesis between hyaline-vascular and plasma-cell CD are suggested by Ly-1b type of mantle-zone immunophenotype in both, by the histologic identification of CD showing plasma-cell and hyaline-vascular histology or a transformation from one type of CD to another in sequential biopsies, and by the recognition of hyaline-vascular CD that produces IL-6 and is associated with systemic symptoms (POEMS syndrome).

Discussion of the paper by Dupin et al at the spring 2000 International Academy of Pathology meeting in New Orleans suggested that the single transformed mantle-zone plasmablasts and the associated plasmablastic microlymphomas lack somatic hypermutation of immunoglobulin variable regions. Because Ly-1b cells also lack hypermutation of immunoglobulin variable regions,18 it would be interesting to know whether the plasmablastic lymphomas or their transformed single-cell counterparts in the mantle zone also lack N-region diversity or restricted V-region gene usage characteristic of Ly-1b cells.19 The lack of somatic hypermutations in the plasmablastic lymphomas would confirm that they are not of germinal center derivation, unlike multiple myeloma, and may offer an explanation for the clinical differences in dissemination and behavior of the plasmablastic lymphomas arising in CD as compared with plasmablastic multiple myeloma. Microdissection experiments of the lambda-restricted plasma cells in PC CD are needed to determine whether they also lack evidence of somatic hypermutation, limited N-region diversity, or restricted V-region usage, as do Ly-1b cells.

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Response:

HHV-8 positive cells in Castleman disease

Dr Menke takes us to task for not quoting his paper1 in which he claims to have discussed the same abnormal mantle-zone cells in Castleman disease (CD) as those that we have described. We were, of course, aware of Dr Menke’s report of Ki-B3 negative mantle-zone cells in CD. He described these Ki-B3 negative cells in all cases of both hyaline-vascular- and plasma-cell-variant CD. It is difficult to ascertain whether

References

he was referring to the entire mantle, as is suggested in figure 1A of his paper; or to isolated cells, and he did not illustrate the cytological features of the cells. By contrast, we have described cytologically distinct HHV-8 positive, IgM-positive plasmablasts occurring within an otherwise normal mantle zone. These cells were restricted to a subset of cases of plasma-cell-variant CD that were HHV-8 positive by PCR. In this context, we saw no reason to quote Dr Menke, and nothing in his letter has caused us to change our view.

To the editor:

**The mouse basophil, a rare and rarely recognized granulocyte**

In a recent article, ultrastructural data were used to identify defects in terminal differentiation of neutrophils in the CCAAT enhancer binding protein family of transcriptional factors knock-out mice (C/EBPε−/−). Peripheral blood of C/EBPε−/− and control mice was obtained from the retro-ocular venous plexus and prepared for electron microscopy, whereas the remainder of the molecular, functional, and biochemical data in this report were obtained using bone marrow cells, peritoneal cells elicited with thioglycollate injections, or the cell line U937 stably transfected with a zinc inducible C/EBPε (p32) expression vector, or empty vector. The electron micrographs illustrated in figure 1A-B of Verbeek et al illustrate a classic mature mouse basophil, not an immature neutrophil as stated, in a C/EBPε−/− mouse. The granules shown are typical for histamine-containing mouse basophil granules, which are less numerous and larger than mature granules in mature mouse eosinophils and neutrophils; basophils do not have secondary and tertiary granules, as do neutrophils, and the electron-lucent structures referred to as glutaraldehyde-extracted secondary granules are typical cytoplasmic vesicles and vacuoles, which are present in basophils from multiple species (see this letter’s Figure). Figure 1C of Verbeek et al shows a classic neutrophil from a control mouse, as indicated in the legend. Whether or not the basophil illustrated in Verbeek et al’s figure 1A-B represents a real increase in this rare granulocyte class in C/EBPε−/− mice cannot be determined from the single cell presented here. Because much of the molecular, functional, and biochemical data for defective terminal neutrophil differentiation are based on studies of bone marrow and elicited peritoneal exudate cells in C/EBPε−/− mice, it would be important to do ultrastructural studies of these cell populations in these knock-out mice and their wild-type controls.

The mouse basophil lineage was defined by ultrastructural analysis, contrasted with developing mast cell, eosinophil, and neutrophil lineages, and reported in 1982 in this journal. These images clearly established the identification and evolution of these common leukocytes.
Ly-1b cells and Castleman disease

David M. Menke