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New Characterization of Infectious Mononucleosis and a Phenotypic Comparison with Hodgkin's Disease

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Recent nucleic acid hybridization studies have implied that Reed-Sternberg/Hodgkin (RS/H) cells are infected with Epstein-Barr virus (EBV) before malignant transformation, and hence, that Hodgkin's disease could develop as a consequence of malignant transformation of an EBV-infected cell. This study is a detailed immunohistochemical and in situ hybridization characterization of the various lymphoid cells in nine cases of infectious mononucleosis (IM), the acute manifestation of EBV infection. The RS/H-like cells of IM were similar in most respects to their morphologically identical counterparts in Hodgkin's disease; they expressed the EBV-encoded protein LMP1, EBV EBER1 transcripts, and CD30 and rarely, if ever, expressed CD45/LCA or T cell markers. Dissimilarities were limited to CD15 negativity and the absence of a collarette of T cells around the RS/H-like cells of IM compared with their Hodgkin's counterparts. Expression of the immortalizing bcl-2 oncoprotein was variable in the RS/H-like cells of IM, as has been demonstrated in the RS/H cells of Hodgkin's disease by other investigators. An apoptosis assay suggested that many apoptotic cells in IM were EBV-infected T cells, in keeping with the previous in vitro observation that IM-derived T cells succumb to apoptosis. Additionally, the apoptosis assay suggested that RS/H-like cells of IM can succumb to programmed cell death, reminiscent of the mummified RS/H cells seen in Hodgkin's disease. The accumulation of evidence suggests that RS/H-like cells of IM are more similar to true RS/H cells than previously recognized. (Am J Pathol 1995, 146:379–388)

Epstein-Barr virus (EBV), the etiologic agent of infectious mononucleosis (IM), has been increasingly implicated in the pathogenesis of Hodgkin's disease. Epidemiological studies have shown that people who have had IM have an increased incidence of Hodgkin's disease,[1,2] and people with Hodgkin's disease have elevated titers of antibodies to EBV before diagnosis of their lymphoma.[3] Recent *in situ* hybridization studies have localized EBV nucleic acid to the malignant Reed-Sternberg/Hodgkin (RS/H) cells in a significant proportion of cases of Hodgkin's disease,[4,5] particularly the mixed cellularity subtype for which 77% of cases are EBV associated.[5] Southern blot hybridization studies have further demonstrated that the viral DNA within the infected cells is monoclonal.[5,6] These data imply that RS/H cells are infected before malignant transformation and, hence, that Hodgkin's disease can result from the transformation of an EBV-infected cell.

The EBV-infected cells in IM include small lymphocytes, immunoblasts, and giant cells that are morphologically identical to the RS/H cells of Hodgkin's disease.[7] Indeed, the presence of RS/H-like cells in lymphoid tissue biopsies from patients with IM may lead to misdiagnosis of Hodgkin's disease. Initial immunohistochemical studies highlighted differences in immunophenotypes between the two types of giant cells; the RS/H cells of Hodgkin's disease are usually CD15 positive and B cell marker negative whereas the RS/H-like cells of IM are CD15 negative[8,9] and B cell marker positive.[9]

The EBV-infected RS/H cell of Hodgkin's disease and the RS/H-like cell of IM are similar, however, in expression of the activation antigen CD30[7,9,10] and the EBV-encoded latent membrane protein (LMP1).[7,11]

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LMP1 confers protection from apoptosis by upregulating expression of the bcl-2 oncoprotein in EBV-infected B cells *in vitro*,[12] but it is uncertain whether this mechanism is operative *in vivo*. Immunohistochemical studies of bcl-2 expression within RS/H cells in Hodgkin's disease have yielded conflicting results.[13–17] To date, bcl-2 expression in RS/H-like cells in IM has not been reported.

This study is a detailed characterization of the lymphoid proliferation in acute infectious mononucleosis. To our knowledge, this is the first study to use the sensitive technique of EBER1 *in situ* hybridization to demonstrate EBV within the RS/H-like cells in IM and the first immunohistochemical study of apoptosis and bcl-2 expression in IM. Our findings demonstrate that the RS/H-like cells of IM are more similar to the RS/H cells of Hodgkin's disease than previously recognized.

Materials and Methods

Patients and Tissue

This study is based on nine lymphoid tissue biopsy specimens (five tonsils, three cervical lymph nodes, and one nasopharynx) from the consultation files of one of the authors (PMB). All cases were morphologically consistent with IM.[8,18] Clinical information and paraffin blocks were provided by the referring pathologists. All cases were subsequently confirmed EBV positive by serology and *in situ* hybridization. Clinical information is provided in Table 1.

Histopathology

All nine formalin- or B5-fixed, paraffin-embedded tissues were examined microscopically with hematoxylin and eosin (H&E) and Giemsa-stained sections. Giant cells with bi- or multi-lobed nuclei and prominent nucleoli were classified as RS-like cells. Their mononuclear variants, Hodgkin cells, were distinguished from immunoblasts on the basis of cell and nucleolar size, with Hodgkin cells being larger and having more prominent nucleoli than immunoblasts. Apoptotic cells were identified morphologically as single shrunken cells with pyknotic nuclei. The major cell type within the interfollicular compartment was noted for each case.

Immunohistochemistry

Immunohistochemical stains were performed on paraffin-embedded tissue sections with a peroxidase-labeled streptavidin-biotin detection system (Dako, Carpinteria, CA) previously described.[19] The monoclonal antibodies used included CD20 (L26), CD43 (Leu22), CD45RO (UCHL-1), CD15 (LeuM1), CD45 (leukocyte common antigen; LCA), CD30, and bcl-2. The polyclonal T cell marker CD3 and a cocktail of EBV LMP1 antibodies (CS1–4) also were used. All of the antibodies were obtained from Dako except CD30, which was obtained from AMAC (Westbrook, ME).

Apoptosis was detected by an assay of endonuclease action described by Gavrieli et al.[20] This assay end-labels nicked DNA by sequential action of ter-

Table 1. *Clinical Data*

Case	Age (*years*)	Gender	Tissue	Clinical presentation	Serology
1	25	M	Tonsil	Airway obstruction IM clinically	Positive monospot
2	15 months	M	Cervical node	IM clinically	Positive monospot
3	80	M	Tonsil	Pharyngitis No suspicion of IM	Positive monospot
4	17	F	Tonsil	IM clinically	Positive monospot
5	26	M	Cervical node	Lymphadenopathy No suspicion of IM	Positive monospot
6	32	M	Tonsil	Airway obstruction No suspicion of IM	Positive specific titers for EBV
7	18	M	Cervical node	Lymphadenopathy No suspicion of IM	Positive specific titers for EBV
8	16	F	Tonsil	Airway obstruction No suspicion of IM	Positive specific titers for EBV
9	38	M	Nasopharynx	Hearing loss No suspicion of IM	Positive monospot, positive specific titers for EBV

minal deoxynucleotidyl transferase (TdT) in the presence of biotinyl-dUTP (GIBCO BRL, Gaithersburg, MD) followed by streptavidin-peroxidase. We modified the technique slightly by using diaminobenzidine to detect the bound peroxidase.

Sensitivity and specificity of the antibody stains were confirmed by using the following tissues for controls: normal lymphoid tissue for CD20 (L26), CD43 (Leu22) CD45RO (UCHL-1), CD3, and CD45 (LCA); an anaplastic large cell lymphoma for CD30; a follicular lymphoma for bcl-2; an EBV-associated Hodgkin's disease for LMP1; normal tissue containing granulocytes for CD15; and normal squamous mucosa for the apoptosis assay.

A quantitative analysis of the immunohistochemical staining of four major cell types was performed on each case of IM with all 10 of the markers described above. The four major cell types analyzed were RS/H-like cells, immunoblasts, interfollicular small lymphocytes, and morphologically apparent apoptotic cells. For each cell type, the proportion of cells staining positively was estimated as none, <25, 25 to 50, 50 to 75, or 75 to 100%. The staining intensity was recorded as weak, moderate, or strong. As the staining pattern of the 3 T cell markers did not vary significantly for a given cell type within each specific case, an average of the 3 markers was reported for each cell type. The proportionate staining per cell type per specific immunostain was then averaged for all nine cases.

Chloroacetate esterase (Leder) stains were performed on a select group of cases (4, 6, and 7) to confirm the presence of neutrophils within the areas of geographic necrosis.

In Situ *Hybridization Studies*

In situ hybridization was performed as previously described[5] with digoxigenin-labeled (Boehringer Mannheim, Indianapolis, IN) riboprobes. Probe templates for EBER1 (RA386)[21] and U6 (RA390) were kindly donated by Richard Ambinder of Johns Hopkins University (Baltimore, MD). Antisense EBER1 probe is complementary to the EBER1 transcript characteristic of latent EBV infection.[22,23] Antisense U6 probe recognizes a ubiquitous cellular transcript[24] that was detected in all nine of the infectious mononucleosis cases examined in this study, confirming that RNA integrity was preserved except in areas of necrosis. The EBER1 staining pattern in the four major cell types was assessed on each of the nine cases of IM as described above for the immunohistochemical stains.

Results

Histopathology

Microscopic examination of the lymph nodes, tonsils, and nasopharynx of the IM patients revealed disruption to total effacement of the normal lymphoid architecture. The interfollicular areas contained an expansive polymorphous proliferation of immunoblasts, lymphocytes, plasma cells, and varying numbers of histiocytes. A mild degree of follicular hyperplasia was present in four cases, but follicles were sparse in the remaining five cases due to the extensive polymorphous interfollicular cellular proliferation. RS-like cells and their mononuclear variants, Hodgkin-like cells, were present in all cases and were most numerous adjacent to foci of geographic necrosis (Figure 1A). A moderate to high number of mitotic figures was always present, and morphologically apparent apoptotic cells were moderate or abundant (with the exception of case 9). The areas of geographic necrosis were often composed of numerous apoptotic cells interspersed in coagulative necrosis (Figure 1B). The presence of neutrophils in the necrotic zones was confirmed on chloroacetate esterase stains.

The morphological findings are summarized in Table 2. Notably, the quantity of RS/H-like cells was

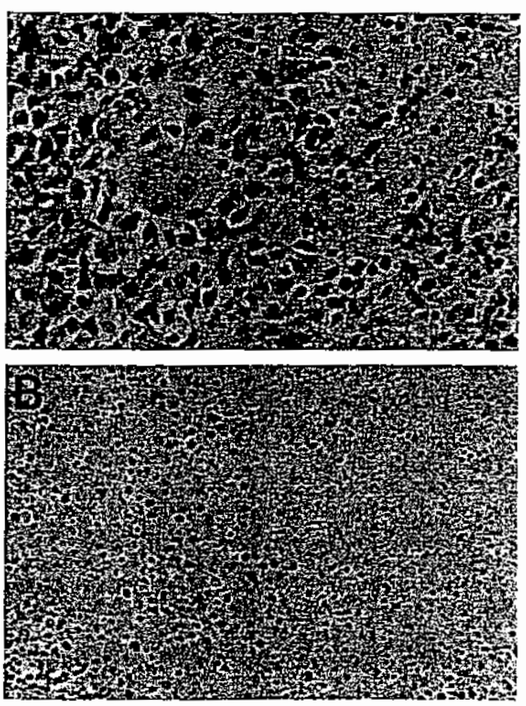

Figure 1. A: *RS/H-like cells are most numerous adjacent to necrosis (upper right).* B: *Necrotic foci contain numerous apoptotic cells (H&E, magnification, ×600 in* A *and ×400 in* B).

Table 2. *Morphological Features*

Case	Age (years)	Tissue	Major cell type	Mitotic rate	Apoptosis, H&E	Necrosis	RS/H-Like cells
1	25	Tonsil	Immunoblasts	High	Abundant	Abundant	Abundant
2	15 months	Lymph node	Immunoblasts	High	Moderate	Rare	Rare
3	80	Tonsil	Lymphocytes Histiocytes	Moderate	Moderate	Abundant	Abundant
4	17	Tonsil	Immunoblasts Lymphocytes	High	Moderate	Moderate	Moderate
5	26	Lymph node	Immunoblasts Plasma Cells	Moderate	Moderate	Moderate	Moderate
6	32	Tonsil	Immunoblasts	High	Moderate	Abundant	Abundant
7	18	Lymph node	Lymphocytes Histiocytes	Moderate	Abundant	Abundant	Abundant
8	16	Tonsil	Lymphocytes	Moderate	Moderate	Moderate	Moderate
9	38	Nasopharynx	Lymphocytes Plasma Cells	Moderate	Rare	Rare	Rare

Mitotic rate is graded as low, moderate, or high. Apoptosis, necrosis, and RS/H-like cells are graded as rare, moderate, or abundant.

directly proportionate to the degree of necrosis in all cases. No correlations were found between the extent of necrosis, the extent of apoptosis, the mitotic rate, or the number of immunoblasts.

Staining Distribution of Common Immunophenotypic Markers

A summary of the immunophenotypic results is found in Table 3. The RS/H-like cells uniformly had strong membrane and Golgi staining with CD30, and many, but not all, of the RS/H-like cells showed moderate to strong membrane and cytoplasmic staining with CD20, consistent with an activated B cell phenotype. The RS/H-like cells consistently failed to stain with CD15 and only rarely stained with CD45/LCA (only three cases demonstrated LCA staining of the RS/H-like cells, and <50% of these cells were positive in all three cases). The RS/H-like cells failed to stain with any of the T cell markers, except in one case in whic <25% of these cells were positive. The RS/H-like cel were noted to lack a discrete peripheral collarette T lymphocytes, unlike that seen surrounding the F cells in Hodgkin's disease. Photomicrographs depic ing the immunophenotype of a typical RS/H-like c are seen in Figure 2.

The immunoblast phenotype was highly variabl proportionate staining varied from <25 to >75 among cases with respect to CD30, CD45/LCA, ar CD20. Overall, the majority of immunoblasts showe moderate to strong membrane staining with CD and, unlike the RS/H-like cells, moderate to stro cytoplasmic and membrane staining with CD45/LC A minority of immunoblasts showed moderate strong cytoplasmic and membrane staining w CD20, and an even smaller minority stained as T cel The immunoblasts consistently failed to stain w CD15.

Table 3. *EBER1 and Immunohistochemical Staining Patterns (Average of 9 Cases), Proportionate Staining Per Cell Type*

	EBER1	LMP1	bcl-2	Apoptosis, TdT	CD30	CD15	CD45/ LCA	CD20	T ce mark
RS/H-Like cells	75–100%	75–100%	25–50% variable*	none	75–100%	none	rare	50–75%	rare
Immunoblasts	50–75%	25–50% variable	<25% variable	none	50–75% variable	none	50–75% variable	25–50% variable	<25%
Interfollicular small lymphocytes	<25%	<25%	25–50%	none	<25%	none	50–75%	<25%	50–7
Morphologically apoptotic cells	<25%	25–50%	<25%	50–75%	<25%	none	25–50%	<25%	25–5

*Variable indicates that staining per cell type varied among cases from <25% to 75% to 100%.

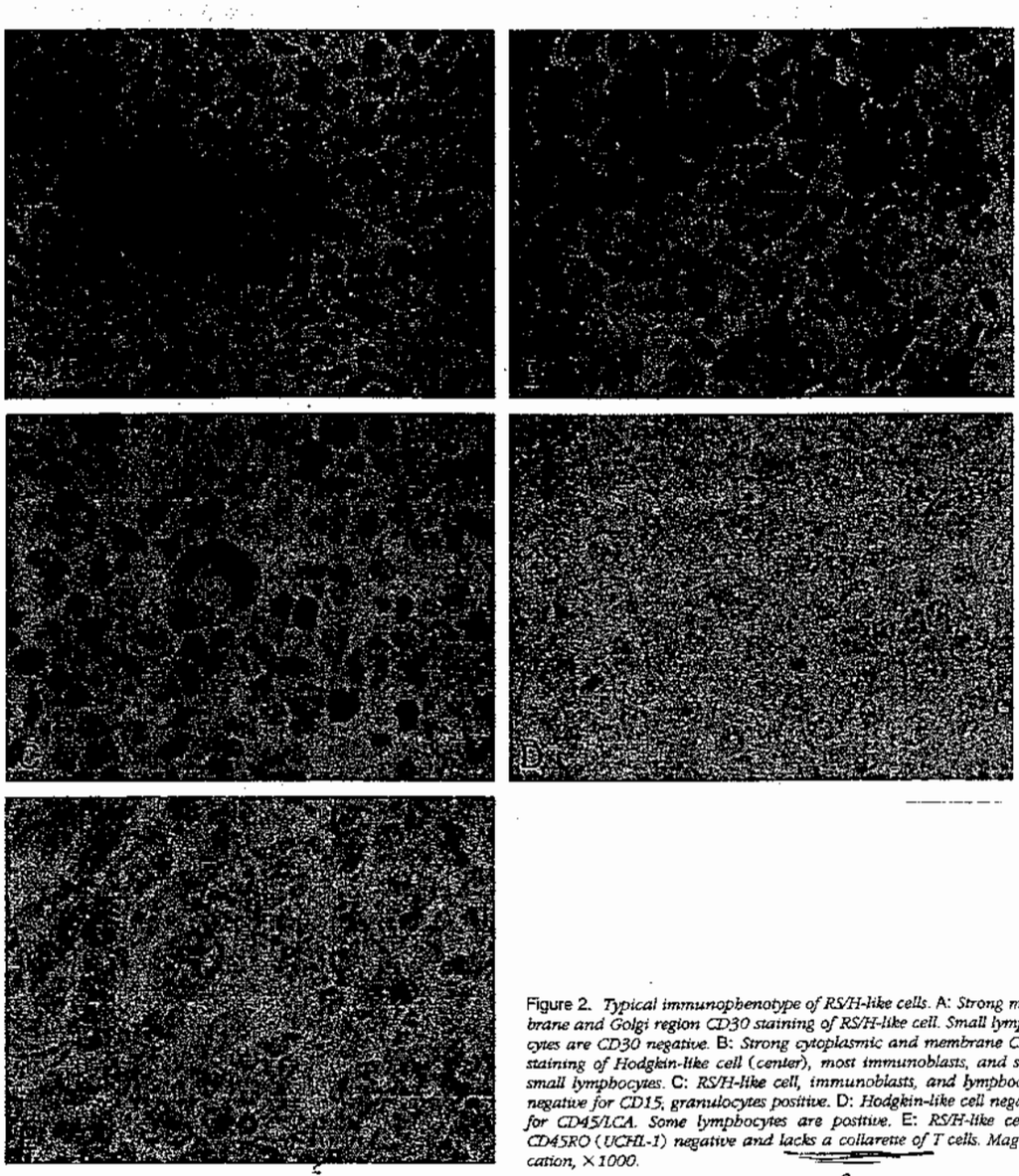

Figure 2. *Typical immunophenotype of RS/H-like cells.* A: *Strong membrane and Golgi region CD30 staining of RS/H-like cell. Small lymphocytes are CD30 negative.* B: *Strong cytoplasmic and membrane CD20 staining of Hodgkin-like cell (center), most immunoblasts, and some small lymphocytes.* C: *RS/H-like cell, immunoblasts, and lymphocytes negative for CD15, granulocytes positive.* D: *Hodgkin-like cell negative for CD45/LCA. Some lymphocytes are positive.* E: *RS/H-like cell is CD45RO (UCHL-1) negative and lacks a collarette of T cells. Magnification, ×1000.*

The majority of interfollicular small lymphocytes had moderate to strong cytoplasmic and membrane staining with CD45/LCA and all three T cell markers, whereas a small minority stained with CD20 and CD30. CD15 staining was consistently negative.

Nearly one-half of the morphologically apparent apoptotic cells demonstrated distinct moderate to strong cytoplasmic staining with CD45/LCA and T cell markers (Figure 3), whereas fewer stained with CD20 or CD30. Individual apoptotic cells were uniformly CD15 negative.

Staining Distribution of EBER1, LMP1, bcl-2, and Apoptosis (Via TdT Assay)

Moderate to strong nuclear staining of EBV-encoded RNA (EBER1) was demonstrated by *in situ* hybridization in all nine cases of IM. The vast majority of RS/H-like cells were positive, the majority of immunoblasts were positive, and only a minority of small lymphocytes were positive (Figure 4A). The EBER1-positive immunoblasts were distributed throughout the paracortex, whereas EBER1-positive RS/H-like

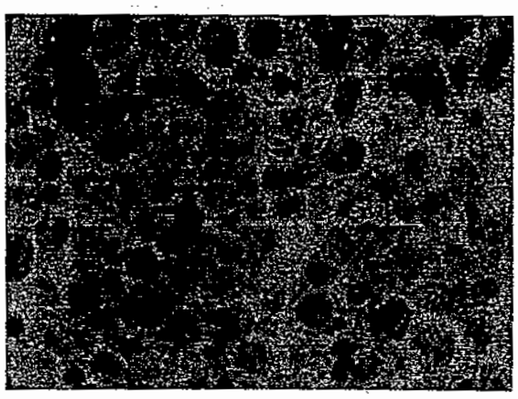

Figure 3. *A zone of necrosis contains CD43-positive T cells and a CD43-positive apoptotic cell (center). Magnification, ×1000.*

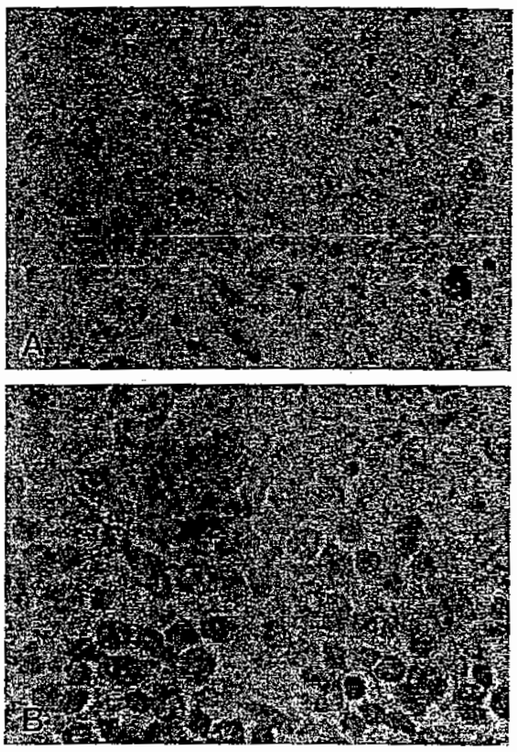

Figure 4. A: In situ *hybridization demonstrates numerous EBER1-positive (purple nuclear staining) RS/H-like cells and immunoblasts, with a small proportion of EBER1-positive lymphocytes (methyl green counterstain). Magnification, ×600.* B: *LMP1 immunostain demonstrates strong cytoplasmic and membrane positivity in an RS/H-like cell at upper left and in an apoptotic cell at lower right. Magnification, ×1000.*

cells were concentrated near foci of necrosis. Strong staining of occasional small cells with round nuclei and condensed chromatin was consistent with the presence of latent EBV in some apoptotic cells. As RNA was poorly preserved in the apoptotic cells as assessed by U6 stains, it is possible that sor apoptotic cells were falsely negative by the EBEI assay.

The vast majority of RS/H-like cells showed stror cytoplasmic and membrane staining of the EB encoded protein LMP1 (Figure 4B), correlating w with their EBER1 and CD30 positivity. Their bcl staining, however, was highly variable; cytoplasm positivity varied from rare cells to the majority of cel and intensity varied from very weak to strong, with tl majority of positive RS/H-like cells staining weakly. N nuclear positivity was identified with the apoptos marker in the RS/H-like cells (Figure 5A).

Immunoblast staining with LMP1 was highly va able from case to case, although the intensity of stai ing was uniformly moderate to strong. There was r apparent correlation between the number of LMP staining immunoblasts and any other immunophen typic or clinical parameter. Only a small minority immunoblasts were bcl-2 positive, except in case 5 which the vast majority were bcl-2 positive. Th apoptosis marker was negative in the immunobla population.

Only a few small lymphocytes were LMP1 positive As expected, a population of small lymphocyte showed moderate to strong cytoplasmic bcl-2 pos tivity in seven cases, corresponding to the norm population of mantle zone memory cells. (Two of th nine cases failed to stain with bcl-2, as determined b lack of staining of this internal control cell population No small lymphocyte positivity was identified with th apoptosis marker.

The majority of morphologically apparent apoptoti cells showed very strong nuclear positivity with th apoptosis marker. Positive cells were mainly smal but occasional large cells that likely represent apop totic RS/H-like cells were also noted (Figure 5A). Th foci of geographic necrosis often contained numer ous positively staining apoptotic cells (Figure 5B) Positive nuclear staining appeared to be specific fo apoptotic cells on the basis of the morphology c these cells and on the pattern of staining within th germinal centers (Figure 5C). In contrast, weak cy toplasmic staining by this assay was not considered to be indicative of apoptosis. Weak cytoplasmic stain ing was found in tingible body macrophages and in terfollicular histiocytes that frequently contained en gulfed apoptotic nuclei (Figure 5A), suggesting tha the cytoplasmic staining was due to degraded DNA from phagocytized cells. Occasional weak cytoplas mic staining was likewise identified in RS/H-like cells Rare scattered small cells present both in the inter follicular areas and within the foci of geographic ne

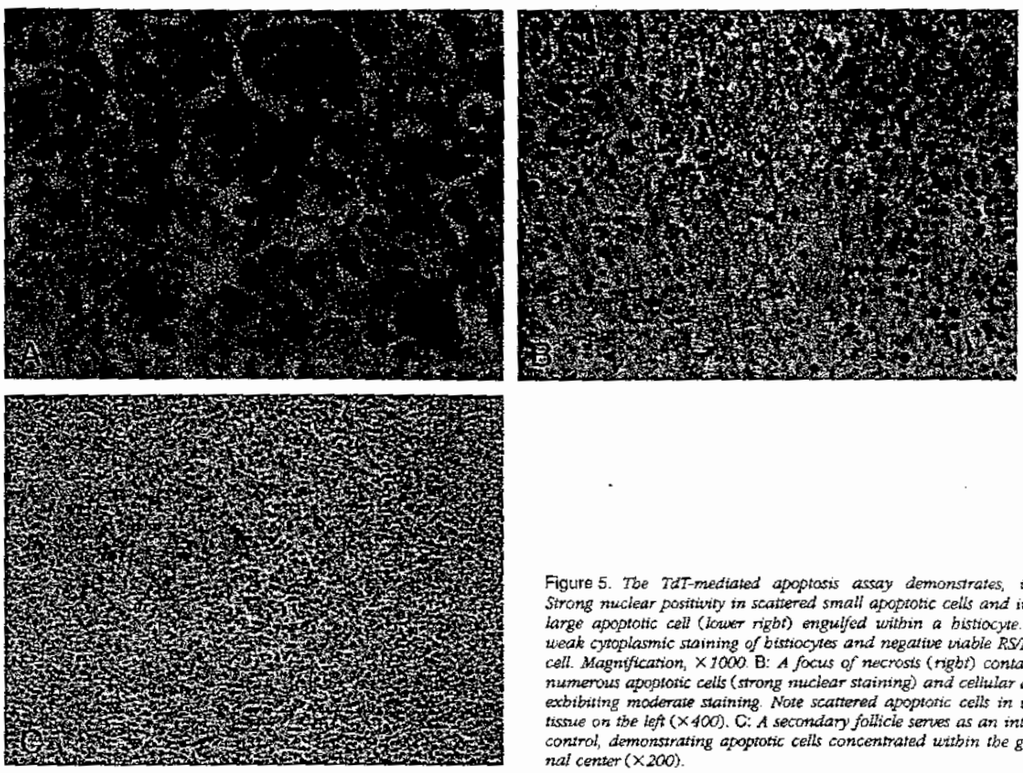

Figure 5. *The TdT-mediated apoptosis assay demonstrates, in* A, *Strong nuclear positivity in scattered small apoptotic cells and in one large apoptotic cell (lower right) engulfed within a histiocyte. Note weak cytoplasmic staining of histiocytes and negative viable RS/H-like cell. Magnification,* ×*1000.* B: *A focus of necrosis (right) containing numerous apoptotic cells (strong nuclear staining) and cellular debris exhibiting moderate staining. Note scattered apoptotic cells in viable tissue on the left* (×*400*). C: *A secondary follicle serves as an internal control, demonstrating apoptotic cells concentrated within the germinal center* (×*200*).

crosis exhibited strong cytoplasmic staining not considered to be indicative of apoptosis,[20] and much debris within the necrotic areas exhibited moderate staining, suggestive of remnants of individual dead cells (Figure 5B). It should be noted that only five of nine cases stained satisfactorily with this apoptosis marker, possibly related to variations in fixation. (The only B-5-fixed case was among those that failed to stain.) A minority of the apoptotic cells demonstrated strong LMP1 positivity (Figure 4B). Surprisingly, rare scattered morphologically apparent apoptotic cells demonstrated a thin rim of cytoplasmic bcl-2 positivity.

Discussion

The morphological findings in these nine cases of IM were similar to those previously described.[18] Notably, the RS/H-like giant cells were usually found bordering foci of geographic necrosis, as described by others.[8,9,18] A new observation in this study was a direct correlation between the quantity of RS/H-like cells and the extent of necrosis. This proportionate and proximate relationship suggests the possibility that cytokines released from the dying tissue contribute to the morphogenesis of these RS/H-like cells. Alternatively, local factors involved in the genesis of such bizarre cells may be responsible for the ensuing necrosis.

Another novel observation was the absence of a collarette of small lymphocytes surrounding the RS/H-like cells. Our immunohistochemical stains confirmed that T cells were not intimately associated with these giant cells in cases of IM, in contrast to the presence of a collarette of T cells surrounding RS/H cells in Hodgkin's disease.[25] The biological significance of the T cell collarette is not understood, but it appears that the collarette is characteristic of malignant RS/H cells.

Previous *in situ* hybridization studies[18,26–28] of IM have not addressed whether EBV nucleic acids are localized to RS/H-like cells. In this study, a sensitive EBER1 hybridization technique revealed that EBV is localized to occasional small lymphocytes, many immunoblasts, and the vast majority of RS/H-like giant cells of IM. Likewise, the vast majority of RS/H-like cells were found to strongly express the EBV-encoded protein LMP1, confirming the findings of

Isaacson et al.[7] In contrast, LMP1 was infrequently expressed in the small lymphocytes of IM and is reportedly absent from the small latently infected lymphocytes in other lymphoid processes,[29,30] suggesting that LMP1 expression is directly associated with cytological features of lymphocyte activation.

Our immunophenotypic findings in RS/H-like cells were similar to those of the three cases reported by Isaacson et al,[7] with the notable exception that only rare RS/H-like cells expressed CD45/LCA in our series. Also, we found that only 50 to 75% of the RS/H-like cells stained with the sensitive B cell marker CD20. As only rare RS/H-like cells stained with any of the T cell markers, this leaves a significant minority of RS/H-like cells negative for both B and T cell markers, analogous to what is seen in the majority of RS/H cells in Hodgkin's disease. Downregulation of CD20 by EBV-infected B cells has been previously demonstrated.[31] Altogether, the findings suggest that RS/H-like cells may represent B immunoblasts that have lost CD45/LCA and are beginning to lose CD20 in concert with a gain of LMP1 protein and acquisition of bizarre cytological features.

Another similarity between the RS/H-like cells in our cases of IM and the RS/H cells of Hodgkin's disease is the variability of bcl-2 oncoprotein expression. Jiwa et al[13] found weak to intense expression of bcl-2 in RS/H cells in 20 of 29 cases of Hodgkin's disease, Zutter et al[15] found weak expression of bcl-2 in RS/H cells in 5 of 9 cases of Hodgkin's disease, and Schmid et al[14] found moderate to intense expression of bcl-2 in all 20 cases of Hodgkin's disease studied. In contrast, two other groups reported the absence of bcl-2 staining in RS/H cells despite positive internal controls.[16,17] Expression of bcl-2 has been shown to be upregulated by LMP1 in EBV-infected B-lymphocytes *in vitro*,[12] potentially protecting these cells from apoptosis. However, variability of bcl-2 staining despite constant strong staining of LMP1 in both IM and EBV-positive Hodgkin's disease indicates this mechanism either is not operative *in vivo* or is only one component of a complex system.

In agreement with previous investigators,[7-9] we found the RS/H-like cells of IM were universally CD15 negative. Thus far, CD15 appears to be a distinguishing marker between the RS/H-like cells of IM and the RS/H cells of Hodgkin's disease, with the RS/H cells in Hodgkin's disease being CD15 positive in the majority of cases (lymphocyte predominant subtype excluded).[32]

IM-derived T cells have been demonstrated to succumb to apoptosis *in vitro*.[33] Our findings suggest that the same mechanism occurs *in vivo* and may be mediated by EBV. Specifically, our data reveal that similar proportions of morphologically apoptotic cells stained with LMP1, CD45/LCA, and the T cell markers, suggesting that the majority of antigen-expressing apoptotic cells were EBV-infected T cells, not B cells or RS-H-like cells. Indeed, the presence of EBV-infected T cells has been demonstrated previously by a double-labeling study of nonneoplastic lymph nodes.[26]

Scattered individual cell apoptosis and geographic necrosis coexisted in all nine cases studied. A TdT-based assay of apoptosis-associated endonuclease activity described by Gavrieli et al[20] confirmed that the morphologically apoptotic small and large cells had biochemical evidence of periodic DNA cleavage characteristic of programmed cell death. Evidence of apoptosis-associated endonuclease activity was also demonstrated in the morphologically defined apoptotic cells present within the geographic zones of necrosis. The possibility that these zones represent sheets of apoptotic cells rather than true necrosis was excluded by chloroacetate esterase stains confirming the presence of neutrophils and by identification of coagulative necrosis. These findings suggest that apoptosis (an energy-dependent cell death process involving endogenous endonuclease activity), and necrosis (a passive cell death process featuring tropism by neutrophils) may coexist within these areas.

Also of interest was the presence of apoptosis-associated endonuclease activity in large cells (Figure 5A). This suggests that RS/H-like giant cells of IM can indeed succumb to programmed cell death. Exactly which factors ultimately determine whether an EBV-infected RS/H-like cell of IM will die or perhaps undergo malignant transformation remain to be determined.

Table 4. *EBER1 and Immunophenotypic Findings in IM and EBV-Associated Hodgkin's Disease*

	RS/H-Like cells in IM	RS/H cells in Hodgkin's disease*	
EBER1	+	+	(Gulley et al[5])
LMP1	+	+	(Pallesen et al[34])
bcl-2	variable	variable	(Jiwa et al[13])
CD30	+	+	(Chittal et al[32])
CD45/LCA	usually −	usually −	(Chittal et al[32])
T cell markers	−	−	(Chittal et al[32])
CD20/B cell marker	usually +	usually −	(Chittal et al[32])
CD15	−	usually +	(Chittal et al[32])
T cell collarette	−	+	(Poppema[25])

*Excluding the lymphocyte predominant subtype of Hodgkin's disease

Table 4 summarizes the immunohistochemical and EBV-specific findings of the RS/H-like cells of IM compared with RS/H cells of Hodgkin's disease. There is a remarkable similarity between these two cell types with respect to most lymphoid markers and LMP1 and bcl-2 expression. As epidemiological and laboratory evidence mounts suggesting a pathogenic role for EBV in a significant proportion of Hodgkin's disease cases, this perspective gains significance. In particular, any differences between RS/H cells and their benign look-alikes might be useful markers of oncogenic events. In this regard, the most striking differences appear to be the nearly complete loss of CD20 and the gain of CD15 and a collarette of T lymphocytes around true RS/H cells in Hodgkin's disease compared with RS/H-like cells of IM. This suggests that any common mechanism underlying the pathogenesis of these two diseases is likely to differ in ways that influence CD15 and CD20 expression and T cell-mediated immune response.

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