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The H-RS–Like Cells in Infectious Mononucleosis Are Transformed Interdigitating Reticulum Cells

SU-MING HSU, MD, and XUN ZHAO, MD

Department of Pathology, University of Texas Health Science Center, Houston, Texas

The lymphoid tissues from patients with infectious mononucleosis or, less frequently, with other reactive conditions may contain Reed–Sternberg (RS)-like cells. These tissues also contain cells resembling the lacunar cells or lymphocytic/histiocytic (L/H) variants, which are present in the lymphocyte-predominant type of Hodgkin's disease. The phenotype of these RS- and L/H-like cells was determined with a large panel of antibodies and lectins. The cells expressed sialylated Leu-M1, Con A, LN-2, and, less frequently, interleukin-1, S-100, and peanut agglutinin receptor. They reacted negatively with two markers for RS cells, Ki-1 and HeFi-1. These RS-like cells were consistently negative for T- and B-cell markers, including immunoglobulins. The markers of the RS-like cells are distinctly different from those in B-immunoblasts, but closely resemble those in interdigitating reticulum cells. It is concluded that interdigitating reticulum cells, when stimulated, can be transformed into lacunar-, L/H-, or RS-like cells. (Am J Pathol 1987, 127:403–408)

IMMUNOBLASTS are generally considered to be either B or T cells. These cells characteristically have one or more large nucleoli. In lymph nodes from patients with infectious mononucleosis (IM), the T-cell zone may be infiltrated with immunoblasts of various sizes. Some of these immunoblasts closely resemble or are indistinguishable from Hodgkin's mononuclear cells and Reed–Sternberg (H-RS) cells, often making it difficult to differentiate IM from Hodgkin's disease (HD).[1,2] Similar H-RS-like immunoblasts may be present singly or in small groups in reactive lymph nodes or tonsils, or in patients with T-cell lymphoma.

We were interested in examining the close resemblance between these cells and H-RS cells. In the present study, we determined the lineage of these immunoblasts by using a large panel of monoclonal antibodies (MAbs). Our aim was twofold: to provide diagnostic criteria for differentiation between IM and HD and possibly to suggest the origin of H-RS cells. Our results indicate that the majority of the immunoblasts in IM are transformed interdigitating reticulum (IR) cells.

Materials and Methods

Tissues

Frozen sections and paraffin sections of B5-fixed lymph nodes from 2 patients with IM were obtained for immunohistochemical and enzyme-histochemical staining. In addition, paraffin sections from five lymph nodes and three tonsils (lymphoid or follicular hyperplasia, nonspecific), all containing scattered Hodgkin-like cells, were used for marker studies. The tissues and sections were processed as described previously.[3–5]

Monoclonal Antibodies and Antisera

The antibodies used and their specificities are summarized in Table 1. Also included in the study are HLA-DR, Lyt 3 (CD2), Leu-1 (CD5), Leu-2a (CD8), Leu-3a (CD4), Leu-4 (CD3), 3A1 (CD7), and OK-T3 (CD3) (T-cell MAbs); OK-T6 (CD1) (T cells and Langerhans' cells); B1 (CD20), B2 (CD21), B4 (CD19), Leu-12 (CD19), and Leu-14 (CD22) (B-cell MAbs); anti-immunoglobulins (Igs); and anti-muramidase.

Establishment of Epstein-Barr Virus (EBV)-Transformed B-Cell Lines

It is known that the antibodies or lectins listed in Table 1 do not react with B lymphocytes and immu-

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Address reprint requests to Dr. Su-Ming Hsu, Department of Pathology, University of Texas, P.O. Box 20708, Houston, TX 77225.

Table 1 — Antibodies Used in This Study and Their Specificity in Tissue Sections

Antibody	Specificity			
	IR cells	Histiocytes	B cells†	T cells
Leu-M1 (CD15)	+(surface)	+(Golgi)	−	−
S-100	+	−(rare+)	−	−(rare+)
IL-1	+	+	−	−
LN-1	−	−	+	−
LN-2	+	+	+	−
Ki-1/HeFi-1 (CD30)	−	−	−	−
1E9	+	+	−	−
Leu-M5 (CD11c)	Rare+	+	−	−
Con A*	+	+	−	−
PNA*	Some+	Some+	−	−
HLA-DR	+	+	+	+/−

*The reactivities of Con A and PNA are based on staining in formalin-fixed, paraffin-embedded tissue sections.

†B cells include immunoblasts.

The table is a summary based on references 7–15.

noblasts in normal and reactive lymphoid tissues, but their reactivity with EBV-transformed B cells is not known. The antibodies anti-Leu-M1, S-100, and interleukin-1 (IL-1) were therefore tested for possible staining with EBV-transformed B-cell lines.

Five EBV-transformed B-cell lines were established from normal human B cells by transformation of the cells with EBV isolated from B95-8 cells. These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. All five cell lines were positive for B-cell markers, such as Leu-14, B1, and Leu-12, and expressed surface and cytoplasmic immunoglobulins.

Table 2 — Phenotypic Comparison of IR Cells, H-RS-like Cells, and H-RS Cells

Antibody	IR cells	H-RS-like cells	H-RS cells
Leu-M1	s+*	s+	s+
S-100	+	+(<10%)	−(rare+)
IL-1	+(50–75%)	+(20–30%)	+
LN-1	−	−	−
LN-2	+	+	+
Ki-1/HeFi-1	−	−	+
1E9	+	+(10–20%)	+(some)
OK-T6†	−	−	−
Leu-M5	+(<10%)	−	−
Con A	+	+	+
PNA	+(10–20%)	+(10–20%)	+(some)
Igs/B cell‡	−	−	−
T-cell§	−	−	−
Acid p-ase	g+	g+(50%)	g+
HLA-DR	+	+	+

*Abbreviations: s, surface; g, Golgi; p-ase, phosphatase.

†Although OK T6 was initially reported to be positive in IR cells, subsequent studies (refs. 6,8,24,25) indicated that IR cells do not express OK T6.

‡B-cell markers included Leu 14, B1, Leu 12, B2, and B4.

§T-cell markers included Lyt 3, Leu 1, Leu 2a, Leu 3a, Leu 4, 3A1, and OK T3

The table is a summary based on the present study and references 6–22.

Staining Procedures

We used the avidin–biotin complex (ABC) immunoperoxidase technique to study the phenotypes of the cells. The staining procedures have been described in detail previously.[4–6] Paraffin sections and cytospin smears were neuraminidase-treated before staining for Leu-M1 antigen.[7,8] Histochemical staining for nonspecific esterase (ANAE) and acid phosphatase (AP) was also performed as previously described.[8]

Results

The majority of the H-RS-like cells (Figure 1) were positively stained by HLA-Dr, LN-2, and Con A, whereas only 10–30% of these cells were positive for PNA and IL-1. The cells were rarely positive for S-100 (Figure 2), and they were negative for LN-1, Igs, lysozyme, α_1-anti-trypsin, and α_1-anti-chymotrypsin.

In all paraffin sections tested, anti-Leu-M1 did not stain H-RS-like immunoblasts or other mononuclear cells, but reacted intensely with granulocytes. However, when tissue sections were pretreated with neuraminidase, anti-Leu-M1 stained most H-RS-like immunoblasts. With neuraminidase treatment, three staining patterns were observed: 1) an intense peripheral (membrane or cytoplasmic and Golgi) reaction, 2) a weak membrane and Golgi reaction, and 3) a Golgi reaction without membrane staining (Figure 3).

Based on their cytologic appearance, these Leu-M1$^+$/LN-2$^+$/Con A$^+$/IL-1$^+$/PNA$^+$ cells can be grouped into three major types: 1) cells containing a prominent nucleolus and having a clumped chromatin pattern, with a few containing two or more nuclei—the so-called H-RS-like cells; 2) cells containing vesicular nuclei and less prominent nucleoli, the nuclei usually being slightly elongated, folded, or lobulated, resembling L/H or lacunar cells; 3) cells with twisted and elongated, nuclei and inconspicuous nucleoli, resembling the IR cells in dermatopathic lymph nodes.

Although they did not stain H-RS-like cells, anti-Igs reacted with plasma cells and immunoblasts located in germinal centers and their mantle zones and, less frequently, in the T-cell zone. The distribution of Leu-M1$^+$ H-RS-like immunoblasts differed from that of Ig$^+$ positive B immunoblasts (Figure 3).

In frozen sections, MAb 1E9 stained a small portion (<20%) of H-RS-like immunoblasts. These

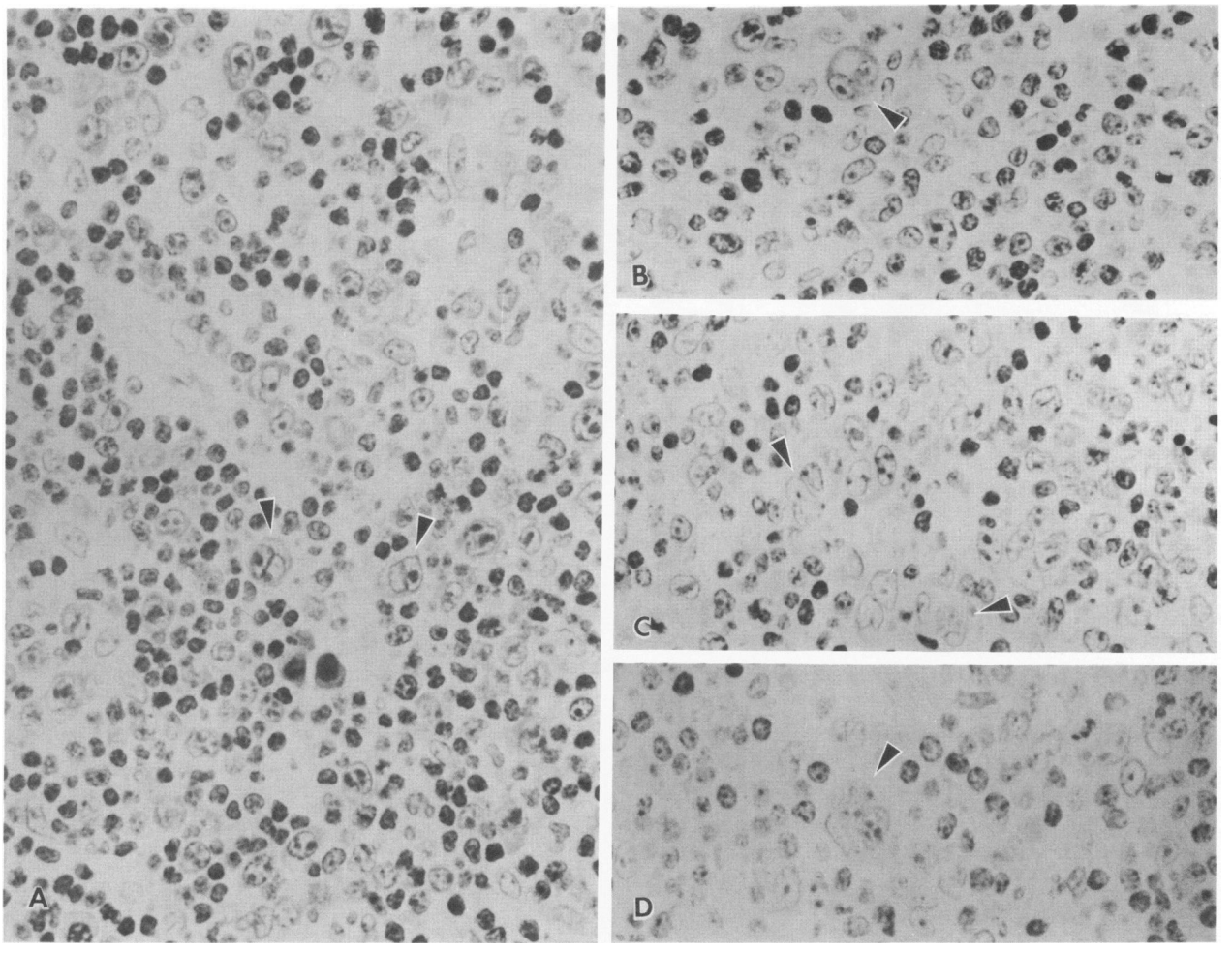

Figure 1—A specimen from a patient with IM. Cells resembling H-RS cells or L/H cells (*arrows*) are illustrated. (×250)

cells were consistently negative for all B- and T-cell MAbs tested. They were also negative for OK-T6, Leu-M5, HeFi-1, and Ki-1. The last two markers were previously known to be associated with H-RS cells in HD. The H-RS-like immunoblasts had a faint acid phosphatase reactivity but were devoid of nonspecific esterase.

The five EBV-transformed B-cell lines were negative for Leu-M1, IL-1, and S-100.

Discussion

It appeared that the H-RS-like immunoblasts in lymph nodes of patients with IM and those in other reactive lymphoid tissues were of neither B- nor T-cell lineage. This conclusion is based on the consistent lack of cytoplasmic Igs, of five B-cell markers, and of seven T-cell markers in these cells. We had found previously, however, that B immunoblasts did not stain well with B-cell MAbs.[4] They can be $B1^-$, $Leu\text{-}12^-$, and $B4^-$, but they are $LN\text{-}1^+$ and Ig^+.[5,9] Virtually all EBV-transformed B-cell lines which we have tested also express LN-1 and surface or cytoplasmic Igs. Furthermore, in paraffin sections of lymphoid tissues, none of B immunoblasts could be stained by anti-Leu-M1, IL-1, or S-100;[9-12] the same is true for the five EBV-transformed B-cell lines tested. Thus, the expression of these three antigens (Leu-M1, etc.) and 1E9 and the absence of B-cell markers (ie, LN-1 and Igs) in H-RS-like cells make a B-cell lineage of these cells quite unlikely.[13,14]

In the present study, we have demonstrated that the H-RS-like immunoblasts are characterized by the expression of sialylated Leu-M1, Con A, LN-2, and, less frequently, of 1E9, IL-1, PNA, and S-100, and that these cells have a faint acid phosphatase reactivity, but express no esterase, lysozyme, or Leu-M5. These findings indicate that the H-RS-like immunoblasts are related to interdigitating reticulum (IR) cells, rather than to histiocytes.[15,16] We had shown earlier

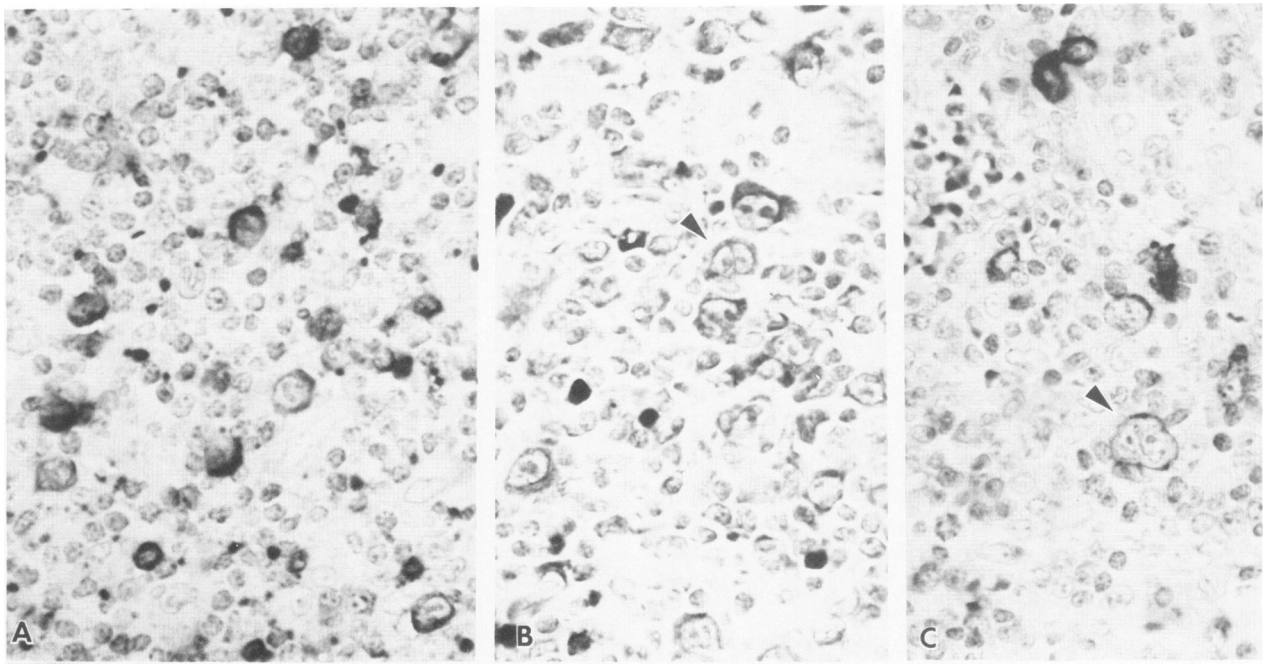

Figure 2—Sections from a patient with IM, in which the H-RS-like cells (*arrows*) are stained for IL-1 (**A**), S-100 (**B**), and PNA (**C**). (×250)

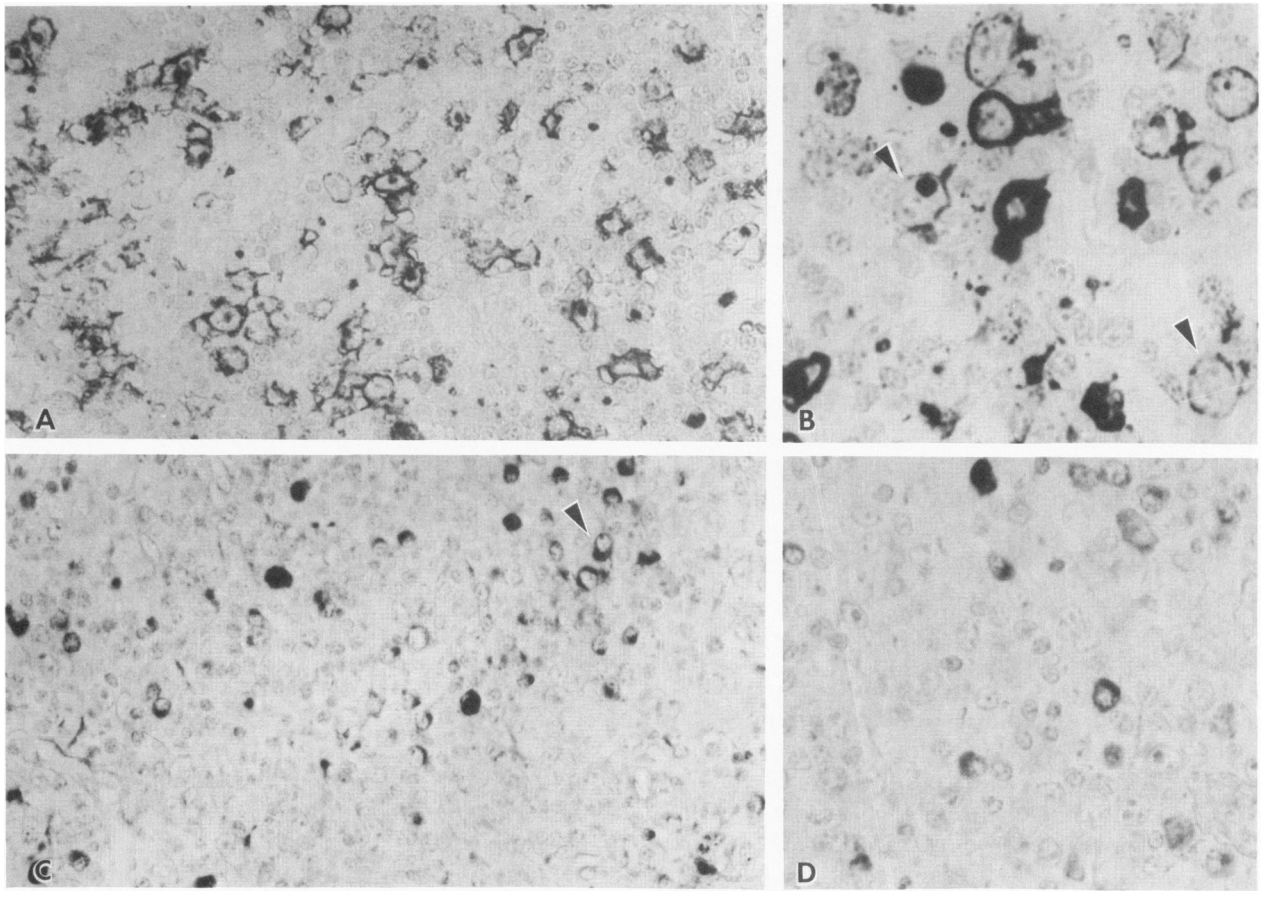

Figure 3—Adjacent sections from a patient with IM, stained for Leu-M1 (**A** and **B**) and Igs (**C** and **D**). The H-RS-like cells (*arrows* in **B**) are stained by anti-Leu-M1, but not by anti-Igs. *Arrow* in **C** indicates an Ig-positive immunoblast. (**A** and **C**, ×250; **B** and **D**, ×400)

that a sialylated surface Leu-M1 antigen is expressed only in IR cells, but not in histiocytes.[7,8]

The variable staining of H-RS-like immunoblasts for Leu-M1, IL-1, 1E9, and S-100 may reflect differences in the status of cellular maturation or differentiation. In the study of dermatopathic nodes, we have noted that activated IR cells were uniformly positive for Leu-M1 and 1E9, but showed variable reactivity with anti-IL-1 and S-100.[7,11,14] The activated IR cells in dermatopathic nodes have elongated and twisted nuclei and less prominent nucleoli than do H-RS-like immunoblasts. In the present study, we noted various cytologic features in the Leu-M1$^+$/Con A$^+$/LN-2$^+$/IL-1$^+$/S-100$^+$/PNA$^+$ cells, ranging from features of typical IR cells to those of lacunar-like or L/H-like cells, and to those of H-RS-like immunoblasts. Both 1E9 and S-100 were absent in the majority of H-RS-like immunoblasts; this may account for the absence of these two antigens in H-RS cells.[13,14]

The close morphologic resemblance and the similar marker expression of the immunoblasts in IM and the H-RS cells in HD are of interest. A possible explanation is that both cells are derived from or related to IR cells, and that one is reactive and the other neoplastic. In previous studies, we proposed on the basis of extensive marker and *in vitro* TPA induction studies[8,17-19] that H-RS cells are derived from IR cells. Although IR cells are generally believed to contain a twisted and elongated nucleus, these cells, when stimulated, may transform into lacunar-like, L/H-like, or immunoblastlike cells. It should also be noted that some activated histiocytes as well as the neoplastic cells from true histiocytic lymphoma can have an immunoblastlike appearance.

The H-RS-like immunoblasts differ from typical H-RS cells in their capacity to sialylate Leu-M1 antigen[8] and in the expression of markers such as HeFi-1 and Ki-1. Thus, in tissues from patients with IM, neuraminidase treatment for removal of sialic acid is needed for a positive anti-Leu-M1 reaction to occur. In contrast, no neuraminidase is required in tissues from patients with HD, except for the lymphocyte-predominant type, in which the L/H cells usually express sialylated Leu-M1 antigen.[20] The use of anti-Leu-M1 and HeFi-1/Ki-1 is therefore useful for differentiating reactive H-RS-like immunoblasts in IM from typical H-RS cells.[21,22]

In summary, the H-RS-like immunoblasts exhibit a phenotype closely resembling that of IR cells, but not that of B or T lymphocytes. We conclude that these cells are likely to be transformed or activated IR cells. IR cells play an important role in cellular immunity,[23] and thus it is not surprising that transformed IR cells are present in large quantities in lymphoid tissues in the presence of viral infections. The morphologic resemblance of these transformed IR cells to H-RS cells supports our hypothesis that H-RS cells are related to IR cells.[16-20]

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