IgE in Reed-Sternberg Cells of Hodgkin’s Disease With Eosinophilia

By Michael Samoszuk

Tissues containing Hodgkin’s disease (HD) of the nodular sclerosis and mixed cellularity subtypes frequently are extensively infiltrated by eosinophils that undergo degranulation. The cause of the eosinophilia in HD remains uncertain, although one possible explanation is provided by the observation that Reed-Sternberg (RS) cells of HD contain messenger RNA (mRNA) coding for interleukin-5 (IL-5), which is a potent stimulator of eosinophil growth and differentiation.

For many years it has been known that patients with HD have elevated levels of serum IgE. Recently, eosinophils and RS cells have been shown to express the CD23 antigen, which is a cell-surface receptor for IgE. Interestingly, eosinophil accumulation and degranulation in cutaneous late-phase reactions have been linked to the presence of IgE within tissues.

Previous immunohistochemical studies of Ig distribution in HD have shown that RS cells contain exogenous, polyclonal Ig. Most of those studies, however, analyzed tissues exclusively for $\kappa$ and $\lambda$ light chains, IgG, IgA, and IgM. An attempt to detect IgE in formalin-fixed samples of HD using polyclonal rabbit antisera and the peroxidase-antiperoxidase method was unsuccessful.

The purpose of this study, therefore, was to use a sensitive and specific immunohistochemical procedure to analyze the distribution of IgE in B-5-fixed tissues involved by HD and to correlate the results, whenever possible, with serum IgE levels and the presence or absence of eosinophils and IL-5 mRNA in the tissues. This report describes the novel observation that HD tissues with eosinophilia often contain substantial deposits of IgE, which are generally not detectable in benign hyperplastic lymph nodes or in non-Hodgkin’s lymphomas (NHLs). The abundant IgE in HD tissues may play a role in producing the infiltration by eosinophils characteristically seen in this disorder.

MATERIALS AND METHODS

Tissues and serum samples. All cases of histologically proven HD newly diagnosed between 1989 and 1991 at UCI Medical Center (Orange, CA) were included in this study. The study group consisted of one case of lymphocyte predominance HD, seven cases of nodular sclerosis HD, and six cases of mixed cellularity HD. The abundant IgE in HD tissues may play a role in producing the infiltration by eosinophils characteristically seen in this disorder.

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IgE IN HODGKIN'S DISEASE

Table 1. Description of Cases and Test Results

<table>
<thead>
<tr>
<th>Diagnosis/History (age/sex)</th>
<th>Tissue Eosinophilia*</th>
<th>Tissue IgE</th>
<th>Eosinophil Count (per μL)</th>
<th>IgE (IU/mL)</th>
<th>IL-5 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRH (7 cases)</td>
<td>Rare</td>
<td>Rare</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>B-cell lymphomas (5 cases)</td>
<td>Rare</td>
<td>Trace in follicles</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>1 LPHD-33/M</td>
<td>None</td>
<td>Traces in follicles</td>
<td>180</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2 NSHD-18/F</td>
<td>Abundant</td>
<td>Abundant</td>
<td>300</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3 NSHD-7/F</td>
<td>Abundant</td>
<td>Abundant</td>
<td>438</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4 NSHD-34/M</td>
<td>Abundant</td>
<td>Abundant</td>
<td>956 (675)</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>5 NSHD-25/M</td>
<td>Abundant</td>
<td>Abundant</td>
<td>NA</td>
<td>Positive</td>
<td>NA</td>
</tr>
<tr>
<td>6 NSHD-19/M</td>
<td>Abundant</td>
<td>Abundant</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>7 NSHD-29/F</td>
<td>Abundant</td>
<td>Abundant</td>
<td>712</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>8 NSHD-38/M</td>
<td>Abundant</td>
<td>Abundant</td>
<td>295</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>9 MCHD-55/M</td>
<td>Abundant</td>
<td>Trace in RS cells</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10 MCHD-48/M</td>
<td>Rare</td>
<td>Trace in RS cells</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>11 MCHD-17/F</td>
<td>Abundant</td>
<td>Abundant</td>
<td>1,283</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>12 MCHD-39/M; AIDS</td>
<td>Abundant</td>
<td>Abundant</td>
<td>415</td>
<td>NA</td>
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</tr>
<tr>
<td>13 MCHD-21/M</td>
<td>Abundant</td>
<td>Abundant</td>
<td>552</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>14 MCHD-32/M</td>
<td>Abundant</td>
<td>Abundant</td>
<td>640 (310)</td>
<td>Positive</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: BRH, benign reactive hyperplasia; LP, lymphocyte predominance; NS, nodular sclerosis; MC, mixed cellularity; NA, not available.

* Tissue eosinophilia was subjectively estimated by routine microscopy and classified as none, rare, or abundant.

IgE immunostaining was subjectively estimated as none, trace, or abundant.

** Immunostaining procedures.** The B-5-fixed tissues were sectioned at a thickness of 3 μm and baked in a dry oven at 56°C overnight. After deparaffinization in xylene and graded alcohols, the tissue sections on glass slides were incubated for 40 minutes with hydrogen peroxide/methanol to block endogenous peroxidase activity.

The washed slides were then incubated for 20 minutes in blocking serum (5% horse serum or goat serum) before application of the primary antibodies. To detect IgE in the tissue sections, the slides from each case were incubated for 30 minutes with 2.5 μg/mL of affinity-purified, mouse MoAb directed against the Fc portion of human IgE (Calbiochem, LaJolla, CA). The slides were then washed, and bound antibody was detected by the avidin-biotin-complex (ABC) procedure, using reagents from Vector Laboratories (Burlingame, CA). After incubation in the chromogenic peroxidase substrate (diaminobenzidine), the slides were counterstained in hematoxylin, intensified with Richard-Allen bluing reagent, dehydrated, and coverslipped before microscopic examination and photography.

For comparative purposes, tissue sections from the cases of HD were also reacted with rabbit polyclonal antibodies directed to human IgG, IgA, and IgM and κ and λ light chains (Dako, Carpinteria, CA). The optimum concentrations of the primary and secondary antibodies were first selected by using a "checkerboard" titration study intended to minimize the background staining of a tonsil control while maximizing the staining signal of B cells. Bound primary antibody was then detected by the peroxidase-antiperoxidase procedure. As a negative control, a tissue section from each case of HD was also incubated with 1:200 normal mouse serum (Coulter, Hialeah, FL) before detection by the ABC procedure as described above.

** RESULTS**

** Immunostaining.** The results of the immunostaining for IgE are summarized in Table 1. In the benign hyperplastic lymph nodes (including two benign lymph nodes from HD patients), IgE was present only in rare plasma cells and in the cytoplasm of follicular dendritic cells (Figs 1 and 2), which are known to express low levels of CD23 antigen.13 In the tonsil, there was also some diffuse staining of the interstitium of the squamous epithelium (not shown). The five cases of B-cell lymphoma had no significant deposition of IgE except for traces in residual follicles.

The HD cases had no detectable staining when incubated with diluted normal mouse serum (Fig 3). Immunostaining of the HD cases with polyclonal antibodies directed against IgG, IgA, and IgM produced highly variable results. Most cases contained obvious IgG within RS cells; cases 6 and 10, however, had relatively weak staining of the RS cells with anti-IgG. RS cells in four cases contained abundant IgA (Fig 4), and IgM was weakly detectable in eight cases. In all cases, there was polyclonal staining for κ and λ Ig light chains.

By contrast, 11 of the 14 cases of HD (including one case of AIDS-related HD) contained readily detectable deposits of IgE within most of the RS cells and variants and on adjacent cells (Figs 5 and 6). Some of these cases also had interstitial and extracellular deposits of IgE as well, but the staining was limited to those microscopic fields that contained histologic evidence of HD. In the single case of lymphocyte predominance HD, IgE and eosinophils were notably absent.

Two additional cases of HD had only traces of IgE in the cytoplasm of some of the RS cells. In one of those cases, eosinophils were readily detectable in the tissues, but the morphology of the tissue sections indicated that the specimen had been poorly fixed. The other case with only traces of IgE was well fixed but was somewhat unusual because eosinophil infiltration was minimal.

** In situ hybridization for IL-5 mRNA.** Three of the cases with abundant eosinophilia and IgE also contained IL-5 mRNA, detectable by a colorimetric in situ hybridization assay. The results were similar to those previously reported and, therefore, are not illustrated.

** Serum IgE levels.** Both patients in whom IgE levels could be measured had moderately elevated IgE levels. In patient 4, the IgE level was 675 IU/mL (equivalent to
Fig 1. Benign hyperplastic lymph node immunostained for IgE. A single plasma cell in the mantle zone of the follicle has strong staining of the cytoplasm, and the follicle has trace staining of the interstitium. Original magnification on all photomicrographs, except Fig 2, is $\times 400$. Diaminobenzidine and hematoxylin counterstain.

Fig 4. Mixed cellularity HD incubated with rabbit polyclonal antibody against human IgA. Some of the RS cells have cytoplasmic staining, indicating the presence of IgA.

Fig 2. Higher magnification of hyperplastic follicle (F) immunostained for IgE. There is membranous staining of some cells and some poorly localizable staining suggestive of follicular dendritic cells. Original magnification $\times 640$.

Fig 5. Nodular sclerosis HD (same as in Fig 3) incubated with MoAb directed against human IgE. The cytoplasm of the RS cells and variants contains obvious staining for IgE.

Fig 3. Nodular sclerosis HD reacted with diluted normal mouse serum. The numerous RS cells and variants have no immunostaining. Note the presence of large numbers of eosinophils with bilobed nuclei.

Fig 6. Mixed cellularity HD incubated with antibody directed against human IgE. The RS cells and Hodgkin’s cells (mononuclear variants) contain cytoplasmic IgE. In addition, there are globular and amorphous deposits of IgE scattered throughout the field.
trolled, immunohistochemical study showed substantial IgE in Hodgkin's disease only in rare plasma cells and in the cytoplasm of follicular cells in 11 of 14 specimens of B-5-fixed, HD tissues. Similar deposits of IgE were not detectable within lymphocyte predominance HD or in B-cell lymphomas without eosinophilia. In seven benign hyperplastic lymph nodes (including two from patients with concurrent HD), IgE was detectable only in rare plasma cells and in the cytoplasm of follicular dendritic cells.

There was only faint staining of the RS cells with antibody directed against IgE in two cases of HD. It is notable that one of these cases had poorly fixed tissue, and our pilot studies suggested that detection of tissue IgE is dependent on the type of tissue fixation. Perhaps the absence of staining for IgE in this case was attributable to poor fixation. In the second case of HD with only trace deposits of IgE within RS cells and variants and on adjacent lymphocytes, staining for IgE, there were very few tissue eosinophils. This finding is consistent with a general correlation between tissue eosinophilia and the presence of IgE deposits; it also suggests, however, that there may be some natural biologic variation in the degree of tissue eosinophilia and IgE deposition in HD.

The immunohistochemical data, in summary, suggest a relatively specific association between IgE and HD, with a histologic distribution of IgE that strikingly resembles the distribution of CD23 previously described in HD. The significance of this observation remains speculative, but a number of provocative possibilities are worth considering.

The first possibility is that the data imply a histogenetic relationship between RS cells and follicular dendritic cells or activated B cells, both of which are known to express the CD23 receptor for IgE. Such a conclusion would be compatible with recent molecular evidence that HD sometimes contains clonal populations of B cells detectable by gene rearrangement analysis.

Although such speculation is tempting, it is premature to draw conclusions about the B-cell origin of the RS cell based solely on the isolated phenotypic observations of IgE deposition and CD23 expression in HD. Consequently, the data in this report should be considered at this time as merely an additional, previously unreported characterization of the in vivo properties of the RS cell and HD tissues.

Another possibility is that RS cells actually synthesize cytoplasmic IgE. Such an interpretation is considered unlikely, however, because other isotypes of Ig within RS cells have been previously shown to be the result of internalization of exogenous Ig rather than the result of endogenous synthesis. Consequently, the cytoplasmic IgE within RS cells is also likely to be the result of phagocytosis of exogenous IgE rather than synthesis of endogenous IgE.

The phagocytosed IgE, in turn, may represent part of the host immune response specifically directed against RS cells or, more likely, circulating IgE that is specifically bound to the CD23 receptor expressed on RS cells and then internalized. The IgE within RS cells is probably not due merely to nonspecific internalization of all plasma IgE because the elevated levels of circulating IgE in the serum of HD patients were still far lower than the levels of IgG. For example, Amlot and Green showed a mean IgE level of 453 IU/mL in the serum of HD patients (normal <150 IU/mL), while the IgG levels were at least 1,000-fold higher.

In this study, two patients had moderate elevations of serum IgE (310 and 674 IU/mL; 0.74 μg/mL and 1.6 μg/mL, respectively) that were still more than three orders of magnitude lower than the normal levels of IgG (12.5 mg/mL). This marked difference in IgG and IgE concentrations would be expected to produce far lower immunostaining intensity for IgE than for IgG if both IgGs were nonspecifically internalized by the same mechanism in RS cells.

Moreover, microscopic fields uninvolved by HD in cases 3 through 14 and the two benign hyperplastic lymph nodes obtained concurrently from HD patients had no evidence of substantial immunostaining for IgE. These results, therefore, strongly indicate that the presence of IgE within RS cells and HD is due to a specific localization of IgE rather than an immunohistologic artifact resulting solely from abnormally high levels of circulating IgE.

It is interesting to note that the CD23 antigen was originally thought to represent a cell-surface marker specific for Epstein-Barr virus (EBV)-infected lymphocytes. Subsequent studies showed that CD23 is actually an Fc receptor for IgE that is transiently expressed at low levels on some B cells before isotype switching and on eosinophils.

In view of recent reports indicating the presence of EBV DNA within HD tissues, it is tempting to propose that the presence of IgE within RS cells may be related to the expression of CD23 surface antigen by EBV-infected RS cells. Additional support for such a hypothesis is provided by the recent demonstrations that EBV-infected B cells and RS cells synthesize IL-5 and mRNA coding for IL-5, thereby suggesting an "expanded role for the (EBV-infected) B cell in the promotion of eosinophilia." Additionally, additional studies will be needed to confirm or refute a pathogenetic relationship between EBV infection, CD23 expression, IL-5 production, and IgE deposition in HD. It is now apparent, however, that IgE, IL-5 mRNA, and EBV are all detectable in HD tissues.

Finally, the presence of IgE deposits within HD tissues raises the intriguing possibility that the tissue eosinophilia in HD is related to the IgE. Such a hypothesis is supported by a recent report that cutaneous late-phase reactions to intradermal allergens are characterized by the deposition of IgE as well as by the accumulation and degranulation of eosinophils. In view of the evidence presented in this and other reports, it now appears that there may be at least two closely related causes of the prominent eosinophilia seen in HD: localized deposition of IgE within the tumor due to production of CD23 receptor, and synthesis of IL-5, perhaps by EBV-infected RS cells.
REFERENCES

IgE in Reed-Sternberg cells of Hodgkin's disease with eosinophilia
[see comments]

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