The Diversity of the Immunohistological Staining Pattern of Sternberg-Reed Cells

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Lymph node tissue of eight cases of Hodgkin's disease of all different subtypes was studied with an immunoperoxidase technique for the detection of immunoglobulin G (IgG), J chain, kappa and lambda light chains, and alpha-1-antitrypsin in different types of Sternberg-Reed cells. It was found that L&H type Sternberg-Reed cells of the nodular lymphocyte predominance type contained IgG, J chain, and one type of light chain per individual cell. It is concluded that these findings indicate that L&H type Sternberg-Reed cells produce IgG and, consequently, are B immunoblasts. Typical and lacunar type Sternberg-Reed cells of mixed cellularity and nodular sclerosis subtypes were found to contain IgG and both types of light chains per individual cell. J chain was absent from these cells and alpha-1-antitrypsin was found in some of them in a paranuclear pattern, comparable to that in histiocytes. It is concluded that these findings exclude the production of IgG by these types of Sternberg-Reed cells and it is suggested that these Sternberg-Reed cells may be related to histiocytes on the basis of the similarity in the staining pattern for alpha-1-antitrypsin.

KEY WORDS: Hodgkin's disease; Sternberg-Reed cells; Immunohistology; J chain; Alpha-1-antitrypsin; Immunoblasts; Histiocytes.

Materials and Methods

Lymph node tissue of eight cases of Hodgkin's disease was routinely fixed in buffered formalin or B5 fixative. Endogenous peroxidase was exhausted with methanol-H2O2 for 30 min (Burns, 1975). All sections were incubated in a 0.1% tyrosin solution in phosphate buffered saline (PBS) (0.05 M, pH 7.6) for 10 min at 3°C. Sections were stained for the different antigens by means of the peroxidase-antiperoxidase (PAP) method as previously described (Poppema et al., 1978). Briefly, the sections were incubated with normal swine serum, diluted 1:10, for 10 min, followed by specific rabbit antisera (anti-human gamma, kappa, or lambda chains, diluted 1:1000, anti-J chain, diluted 1:100, and anti-alpha-1-antitrypsin, 1:1000) for 60 min, and finally by a swine anti-rabbit Ig antiserum, diluted 1:40, and PAP, diluted 1:50. Each incubation was for 30 min. The antiserum were obtained from Dakopats (Copenhagen, Denmark) except for the rabbit anti-J chain antiserum, which was obtained from Nordic (Tilburg, the Netherlands). Control sections were treated with normal rabbit serum, diluted 1:20, in place of specific antiserum. Washings between incubations were performed in three changes of PBS (0.05 M, pH 7.6) for 15 min. The peroxidase was stained with diaminobenzidine-

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tetrahydrochloride and H2O2 according to Graham and Karnovsky (1966).

A double staining technique for the demonstration of two different antigens in the same section was performed as described previously (Poppema et al., 1978), except for the omission of the treatment with acid buffer (Sternberger and Joseph, 1979). The second antigen was stained with 4-chloronaphthol and H2O2 according to Nakane (1968).

Results

In all cases studied a reliable staining was obtained, with clear staining of positive cells and minimal or absent background staining. Sections incubated with normal rabbit serum in place of specific antiserum showed no staining. A large proportion of the Sternberg-Reed cells and their variants stained positively for IgG in all cases. The intensity of the staining was variable, but always less than that of the reactive plasma cells. In most L&H type Sternberg-Reed cells the staining was more coarsely granular or globular and often restricted to the paranuclear or parannuclear area. Sections stained for kappa or lambda light chains showed similar numbers of positively staining Sternberg-Reed cells, when compared with each other or with the sections stained for IgG. However, in the case of nodular lymphocyte predominance type the majority of Sternberg-Reed cells stained for kappa, and only a few for lambda, light chains.

The double staining for kappa and lambda light chains in the same section of the case of nodular lymphocyte predominance showed that the majority of the L&H type Sternberg-Reed cells stained brown for kappa and a few cells stained bluish-grey for lambda. In all other cases no Sternberg-Reed cells could be demonstrated that were positive for only one light chain. The plasma cells in these cases showed brown staining for kappa and bluish-grey staining for lambda light chains in a ratio of about 2:1.

Staining for J chain revealed a small number of positive plasma cells in each case, but the majority of plasma cells was completely negative. In the nodular lymphocyte predominance case a distinct, granular staining for J chain was present in L&H type Sternberg-Reed cells in a pattern comparable to the staining for IgG (Figure 1). In all other cases the Sternberg-Reed cells were devoid of staining for J chain, despite strong staining for IgG.

Alpha-l-antitrypsin was present in some histiocytes, especially some epithelioid cells, in a paranuclear localization and in other histiocytes in a more diffuse pattern. In the Sternberg-Reed cells of the cases of nodular sclerosis and mixed cellularity, the staining pattern was similar to that in the histiocytes. Some Sternberg-Reed cells showed a diffuse, granular positivity comparable to the staining for IgG, but in other cells a paranuclear staining was observed (Figure 2). The L&H type Sternberg-Reed cells in the nodular lymphocyte predominance case did not stain for alpha-l-antitrypsin. The Sternberg-Reed cells in the lymphocyte depletion case also did not stain for alpha-l-antitrypsin, although they were strongly positive for IgG. The staining results in the different subtypes are summarized in Table 1.

Discussion

The finding that all immunoglobulin-containing Sternberg-Reed cells in cases of nodular sclerosis, mixed cellularity, and lymphocyte depletion stain for both kappa and lambda light chains confirms some earlier reports (Landass et al., 1977;
Kadin et al., 1978; Poppema et al., 1978), but differs from a number of other studies in which it was concluded that Sternberg-Reed cells may contain one type of light chain (Garvin, 1974; Taylor, 1974; Papadimitriou et al., 1978). This difference is probably the result of at least two factors. Some studies were performed on material of uncontrolled fixation and it is not unusual to get unreliable staining results in such cases. This problem can be overcome by a large extent by trepsinization of the sections before the staining (Mepham et al., 1979; Hauzter et al., 1980). The other problem is the great difficulty that is encountered in the identification of individual cells in serial sections. This can be overcome by double staining for both light chains in the same section and this procedure enabled us to conclude that in these types of Hodgkin's disease no Sternberg-Reed cells were present with only one type of light chain. The absence of J chains from Sternberg-Reed cells in these types of Hodgkin's disease was first described by Isaacson (1979) and was confirmed by this study. In normal lymphoid tissue J chain is found in IgM-producing cells, in some IgA-producing cells, and in immature IgG-producing cells (Korsrud and Brandtzaeg, 1980). It was demonstrated in normal immunoblasts irrespective of the class of immunoglobulin synthesized (Brandtzaeg, 1976) and in a variety of immunoglobulin-producing B-cell lymphomas (Isaacson, 1979). Mature, IgG-producing plasma cells, however, have no demonstrable J chain and this correlates well with the absence of J chain in the majority of the plasma cells in these cases of Hodgkin's disease. The data indicate that production of IgG by Sternberg-Reed cells of nodular sclerosis, mixed cellularity and lymphocyte depletion cases is very unlikely and that the presence of IgG in these cells therefore cannot be taken as evidence for a B-cell origin.

The L&H type Sternberg-Reed cells of nodular lymphocyte predominance type of Hodgkin's disease showed different staining characteristics. The IgG was present in coarse granules, which were often perinuclear or paranuclear localized, resembling the type of staining found in immunoblasts and plasma cells. In these Sternberg-Reed cells only one type of light chain per individual cell was demonstrated and in the case studied there was a strong preponderance of kappa light chain positive cells. These findings fully confirmed our previous study on L&H type Sternberg-Reed cells (Poppema et al., 1979). In addition, it was demonstrated that J chain was present in the cytoplasm of these cells and it can therefore be concluded that L&H type Sternberg-Reed cells do produce immunoglobulin and consequently are B immunoblasts.

The presence of alpha-1-antitrypsin in Sternberg-Reed cells in the mixed cellularity and nodular sclerosis cases can be explained by a number of mechanisms. Its presence, together with IgG and albumin, was originally taken as an argument for

| Table 1. Immunohistological staining results in Sternberg-Reed cells of the different subtypes of Hodgkin's disease |
|--------------------------------------------------|-----|-----|---------|-----|
|                          | IgG | κ and λ | κ or λ | J chain | α-1-αT |
| Lymphocyte predominance, nodular (1 case)        | +   | +       | +       | +      | -      |
| Nodular sclerosis (4 cases)                       | +   | +       | -       | -      | +      |
| Mixed cellularity (2 cases)                       | +   | +       | -       | -      | +      |
| Lymphocyte depletion (1 case)                     | +   | +       | -       | -      | -      |
a nonspecific uptake of serum proteins from the environment (Poppema et al., 1978). Other possibilities are a specific uptake of alpha-l-antitrypsin-protease complexes by reticuloendothelial cells, as described by Ohlsson (1971), and ultimately it is possible that alpha-l-antitrypsin is produced by these cells. The paranuclear localization in some Sternberg-Reed cells and epithelioid cells could be taken as an argument for production of alpha-l-antitrypsin, although pinocytosed material also would be transported to that area. Whatever the mechanism of the paranuclear accumulation of alpha-l-antitrypsin in Sternberg-Reed cells is, the presence of this protease inhibitor in histiocytes and its absence from other lymphoid cells may be sufficient to permit its use as a marker for the histiocytic origin of cells, as was done in a study on primary gastrointestinal lymphoma (Isaacson et al., 1979). If alpha-l-antitrypsin is accepted as a marker for histiocytes, this would imply that Sternberg-Reed cells of mixed cellularity and nodular sclerosis types of Hodgkin’s disease are derived from histiocytic cells.

In conclusion it can be said that the use of the immunoperoxidase technique permits the recognition of different types of Sternberg-Reed cells in the different subtypes of Hodgkin’s disease. The L&H type Sternberg-Reed cells that are present in the nodular lymphocyte predominance subtype bear all the markers of B immunoblasts. The typical and lacunar type Sternberg-Reed cells of mixed cellularity and nodular sclerosis types have staining characteristics that are compatible with a histiocytic origin. Finally, it should be emphasized that cases with a predominance of lymphocytes which show easily recognizable typical or lacunar type Sternberg-Reed cells should be classified as mixed cellularity or nodular sclerosis subtype (Lukes and Butler, 1966) and that these cases have features which are different from those of the nodular lymphocyte predominance subtype (Poppema et al., 1979a,b,c).

Literature Cited


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