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Correspondence

CD40 Antigen Expression on Reed-Sternberg Cells

A Reliable Diagnostic Tool for Hodgkin's Disease

To the Editor-in-Chief:

CD40 is a member of the tumor necrosis factor/nerve growth factor receptor family, showing a significant homology to the Hodgkin's disease (HD)-associated antigen CD30,[1] and capable of transducing growth signals in a number of cell types. It has been recently shown that CD40 may play an important role in the regulation of Reed-Sternberg (RS) cell expansion and in the contact-dependent interactions of these cells with cytokine-producing T lymphocytes.[2,3] Although CD40 has been extensively studied on B-cell populations, certain carcinomas, and myeloma plasma cells, little was known about its expression in other tumor cell types such as the putative neoplastic cells of HD and related malignancies, including CD30 positive anaplastic large cell (ALC) lymphomas.

In the January 1994 issue of the *American Journal of Pathology*, O'Grady et al[4] reported on the expression of CD40 in 37 cases of HD and 23 cases of non-Hodgkin's lymphoma (NHL) other than CD30-positive ALC lymphomas, examined by paraffin-section immunohistochemistry using the BB-20 monoclonal antibody (MAb). O'Grady et al[4] detected high levels of CD40 expression on RS cells in 26 out of 37 HD, but only in 2 out of 12 B-cell NHL, and 1 out of 11 T-cell NHL cases. The authors concluded that there is probable overexpression of CD40 in HD and suggested that dysregulation of CD40 expression may play a role in the pathogenesis of this disease. We would like to make a few comments on this hypothesis based on our own experience.

Recently, we have evaluated for CD40 expression a series of 195 lymphoma samples, including 171 cases of HD (16 lymphocytic predominance, 109 nodular sclerosis, 41 mixed cellularity, 5 lymphocytic depletion) and 24 cases of CD30-positive ALC lymphomas, by immunostaining of paraffin-embedded sections. Only CD30-positive ALC lymphomas of T- and "null"-cell types were selected for study according to the stringent criteria for this diagnosis provided in the recent proposal from the International Lymphoma Study Group.[5] All surgical specimens were fixed in Bouin solution or formalin and embedded in paraffin. Deparaffinized sections were immunostained by using anti-CD40 MAb 89 and the alkaline anti-alkaline phosphathase (APAAP) method. Briefly, the results of this investigation on a large case series indicated that CD40 was strongly expressed on most RS cells and variants in 100% (171/171) of HD cases, irrespective of their antigenic phenotype (T, B, "null") and type of tissue fixation. Conversely, CD40 was immunodetected only in 5 out of 24 (20.8%) CD30-positive ALC lymphoma cases. Anti-CD40 antibody stained RS cells with a highly distinct pattern (intense membranous with a strong dot-like pattern in the paranuclear area) (Fig. 1), which was easily recognizable and allowed a confident separation of HD from CD30-positive ALC lymphomas. In this latter tumor, only a fraction of malignant cells were usually labeled, and they displayed in general a lower surface density of CD40 as compared with RS cells. Moreover, cytoplasmic staining or dot-like paranuclear positivity were rarely appreciated. In a different contemporary study employing the anti-CD40 MAb M2, RS cells were found to express surface and cytoplasmic CD40 in 13 out of 13 HD cases (9 nodular sclerosis, 4 mixed cellularity),[2] and the staining intensity showed high-level expression in comparison with CD40 staining of tonsil B-cells.

Overall, these findings are in apparent contrast with the data of O'Grady et al[4] indicating that a sig-

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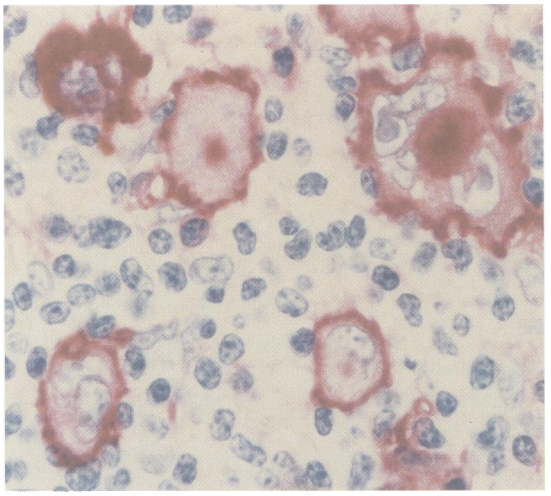

Figure 1. *Hodgkin's disease, nodular sclerosis subtype. RS cells show a distinct pattern of staining for anti-CD40 antibody 89: a strong membrane staining is associated with a dot-like cytoplasmic positivity. Bouin-fixed paraffin-embedded tissue section, APAAP immunostaining, hematoxylin counterstain, ×630.*

nificant fraction (about 30%) of HD cases do not express CD40 as detected by MAb BB-20.[4] Such discrepancy may be explained in part by an exquisite sensitivity to fixation procedures of the CD40 epitope recognized by the MAb BB-20. The use of different immunodetection procedures, i.e., APAAP staining[3] *versus* ABC,[4] and the use of frozen[2] *versus* paraffin-embedded tissue sections should be also taken into account. In general, CD40 is found on most stages of B-cell differentiation (exception: plasma cells), malignant B-cells in lymphomas, leukemias, and virally transformed B-cells (summarized in ref. 6). It is of interest to notice that O'Grady et al[4] only detect CD40 expression in 2 out of the 12 B-cell NHLs investigated. Detailed biochemical studies regarding the epitope mapping, affinity, or association/dissociation rates have not been performed for the panel of CD40 MAbs. Further studies have to show which of the CD40 MAbs are best used for the analysis of CD40 expression in primary tissues, but it seems likely that the MAb 89 and M2 are more sensitive than BB-20.

Our findings indicate that CD40 expression is of high value in the identification of RS cells in Bouin or formalin-fixed paraffin embedded material. CD40 expression has important diagnostic application for distinction between CD30-positive ALC lymphomas and HD. Differences in CD40 reactivity and the unique pattern of staining of RS cells might be additional features useful in differentiating HD from CD30-positive ALC lymphomas. Further studies have to confirm that the absence of CD40 could represent a further tool to exclude HD for the differential diagnosis of HD *versus* ALC lymphoma. Taken together, CD40 expression of RS cells is an important biological and reliable pathological marker for HD with all of the cases positive. We therefore propose the application of anti-CD40 antibodies for routine identification of RS cells and for the diagnosis of ambiguous cases of HD.

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