To the Editor:

In a recent letter, Tao et al.' maintain that the neoplastic cells of the CD56− nasal T/natural killer (NK) cell lymphomas are not immunoreactive for CD3 either using Leu4 or polyclonal (rabbit) anti-CD3c antibody, contrasting with the positive results observed in most cases by Suzumiya et al.\(^1\) They stress the need for combining EBER in situ hybridization with immunohistochemistry or double immunohistochemistry for accurate identification of the immunophenotype of nasal T/NK cell lymphomas.

In our experience of interpretation of frozen section immunohistochemistry on more than 80 cases of nasal lymphomas, we have found that interpretation of antigen expression on the neoplastic cells is generally not difficult if one compares the pattern of staining, the percentage of positive cells, and the size of positive cells in the slides stained with the different antibodies.\(^5\) Furthermore, some cases are predominated by sheets of large cells, and interpretation should be very easy. We have found the immunophenotype of CD2+ CD3(Leu4)− CD56+ to be most common.\(^6\) In such cases, CD2 reactivity often results in sheets of positive cells. A corresponding field stained with Leu4 (CD3) shows a much lower proportion of positive cells, often isolated or in small groups, which represent residual T cells. Furthermore, the Leu4+ cells are smaller than most cells that are CD2+.\(^7\) (Fig 1). Staining with CD56 also often shows sheets of positive cells. A rough estimation often indicates that the total number of Leu4+ cells (reactive cells) and CD56+ cells (neoplastic cells) equals that of the total CD2+ cells. In those occasional cases in which the neoplastic cells are masked by a prominent component of reactive T cells, careful comparison of the different slide preparations and attention to the cell size will often permit a correct interpretation to be made. Although Tao et al.' emphasize surface versus cytoplasmic staining for CD3, we find it difficult, if not impossible, to distinguish between the two in frozen sections because the rim of cytoplasm is so thin.

Our recent study comparing the staining with Leu4 (CD3) on frozen sections and rabbit anti-CD3c antibodies on paraffin sections can perhaps clarify the controversial issue of CD3 staining in CD56− T/NK cell lymphomas.\(^5\) Among 18 cases of CD56− nasal T/NK cell lymphomas that are negative for Leu4 in frozen sections, 17 (94%) are stained by anti-CD3c in paraffin sections.\(^8\) The reactivity can be easily recognized to be on neoplastic cells because of the atypia, irregular foldings, and/or large size of the nuclei (Fig 1D). The staining is often in the perinuclear space.

Ohno et al.' also recently reported almost identical results, with six of the eight Leu4− CD56− nasal lymphomas being found to be CD3−.\(^9\) These results are thus in agreement with those of Suzumiya et al.,\(^2,3\) who reported that eight of nine CD56− nasal lymphomas were CD3−. Strickler et al.'\(^10\) and van Gorp et al.'\(^11\) have similarly reported CD3+ positivity in ∼90% of nasal T− cell lymphomas. The failure of Tao et al. to detect convincing CD3c expression in paraffin sections of nasal T/NK cell lymphomas may be related to an inadequate antigen retrieval procedure, because staining with rabbit anti-CD3c antibody is strongly influenced by effectiveness of antigen retrieval.\(^8,12\) Alternatively, they might have missed the CD3+ positivity; in their color figure, a brown blush appears to be present around some of the dark blue-staining nuclei.

We cannot explain the high Leu4 positivity in nasal CD56+ lymphomas in the series of Suzumiya et al., a finding that we and others have not found in this group of lymphomas.\(^4,13\) For cases 1 and 5, the sum total of CD56+ and Leu4− cells is still around 100%,\(^7\) and thus the Leu4+ cells could still have been reactive T cells.

The discordance of staining between Leu4 (CD3) and polyclonal anti-CD3c antibodies in nasal T/NK cell lymphomas may be explained by the expression of cytoplasmic CD3c only; Leu4 antibody will not give a positive reaction because it apparently recognizes an epitope formed by interaction of the various chains of the complete CD3 molecule.

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Fig 1. Prototypical case of nasal T/NK cell lymphoma. (A) Staining with T11 (CD21) shows numerous closely-packed positive cells. The positive cells are smaller than most of the CD2+ cells, and they represent residual or reactive T lymphocytes. (B) Staining with Leu4 (CD3) shows only scattered positive cells. (C) Staining with NKH1 (CD56) also shows sheets of positive cells. (D) Immunostaining with polyclonal anti-CD3c antibody shows that the neoplastic cells give a positive reaction in the perinuclear space. (Immunoperoxidase staining on frozen section, no counterstain; original magnification × 300.)


8. Chan JKC, Tsang WYW, Ng CS, Pau MY: Discordant CD3 expression in lymphomas as studied on frozen and paraffin sections. Hum Pathol 26:1139, 1995


Response

The majority of nasal lymphomas were previously considered to be T-cell lineage because they expressed CD3. However, together with our results, many researchers reported that nasal lymphoma cells expressed CD3e, CD2, and CD56, but have low frequency of T-cell receptor (TCR) rearrangement. Therefore, it is feasible to postulate that nasal lymphomas having such characteristics are of NK cell lineage. In this context, we are glad to know that Chan et al agree with our results that nasal lymphoma cells expressed CD3e.

One minor point to be solved is the expression of CD3 (Leu4; Becton Dickinson, San Jose, CA) on nasal lymphomas. Although we were able to show clearly positive staining of Leu4 in nasal lymphomas in our photograph, Chan et al mentioned in their letter that CD56+ nasal T/NK lymphomas did not express CD3 (Leu4). Subsequently, we performed immunohistochemical examinations using simultaneously Leu4 and OKT3 (Ortho, Raritan, NJ) that recognize CD3e and e6 and found that the staining patterns with OKT3 were similar to those with Leu4 except for the relatively lower positivity than that with Leu4. Related to our results, a few possible cases having simultaneously expressing CD3e or e6 and CD56 without TCR rearrangement were reported. Because we have identified nasal lymphoma cells as NK cell, but not T cell, lineage primarily based on the lack of TCR rearrangement, the stainability of CD3 (Leu4) is not critical for this identification. Because the estimation of CD3 positivity on frozen sections is troublesome in some cases, further study of double-labeling method using Leu4 and CD56 would solve this problem.

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Clarification of CD3 immunoreactivity in nasal T/natural killer cell lymphomas: the neoplastic cells are often CD3 epsilon+ [letter; comment]

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