To the Editor:

Suzumiya et al recently described 9 cases of nasal lymphomas that were CD56+CD2+. Based on T-cell receptor (TCR) gene rearrangement studies and immunohistochemical staining with two CD3 markers (Leu 4 and rabbit anti-CD3ε), they classified the cases into three distinct groups: TCR-CD56+CD3(Leu4)+CD3ε+, TCR-CD56-CD3(Leu4)+CD3ε+, and TCR-CD56-CD3(Leu4)+CD3ε+. They further hypothesized that the cellular origin of these groups might correspond to activated adult natural killer (NK) cells, fetal NK cells, and T cells, respectively. A single immunohistochemical technique was used by Suzumiya et al in establishing the phenotype of the tumor cells. In our experience, it can be difficult to distinguish reactive lymphocytes from neoplastic cells unequivocally based on morphologic features or single immunohistochemistry alone in this type of nasal lymphomas because of its pleomorphic feature, which may lead to misinterpretation of the tumor phenotype.

We reported previously that nasal lymphomas of T cell phenotype were mostly TCR- (germline configuration for both TCR and Ig genes); only 1 of 8 cases had a rearranged TCRγ chain gene. Although CD3 positivity is sometimes observed in CD56+ nasal lymphomas, we have pointed out that it is very difficult to ascertain whether these CD3+ cells are tumor cells or reactive T lymphocytes before a dual-labeling study is performed. We have shown clonal Epstein-Barr virus (EBV) in nasal lymphomas, which supports the idea that EBV+ cells represent a neoplastic population. Similar results have been obtained by other groups. By combining EBER in situ hybridization (ISH) with immunohistochemistry (IH) for cellular markers and double IH, we recently identified the lineage of EBV+ cells in a series of nasal lymphomas. The phenotype of the EBV+ cells in each case corresponded to the genotype of the tumors, which further illustrated the close association between EBV and the neoplastic cells. Interestingly, it was noticed that in two cases of TCR-CD56+CD2+ nasal lymphomas, 20% to 50% of tissue cells were CD3ε+ (Leu-4 or anti-CD3ε). However, virtually all the CD3ε (or leu-4) cells were EBV+, whereas the EBV+ cells were virtually all CD3ε (or leu-4)- (Fig 1). These CD3ε (or leu-4)+ cells were, therefore, most likely background-reactive T cells. The pattern of the immunostaining with rabbit anti-CD3ε or leu-4 in these cases was typical surface membrane staining. By contrast, a nasal lymphoma of true T-cell lineage confirmed by gene rearrangement studies showed CD3(Leu-4 or anti-CD3ε)+EBV+ tumor cells.

Suzumiya et al did not record the percentage of CD3+ cells in their tumors, nor have they illustrated unequivocal intracytoplasmic rather than membrane staining of CD3 (Leu-4 or anti-CD3ε) in their cases. Freshly isolated adult NK cells express a very low level of cytoplasmic CD3ε; activated adult NK cells express detectable level of cytoplasmic CD3ε; whereas fetal NK cells express cytoplasmic CD3ε, CD3γ, and CD3δ whose complex can be recognized by CD3 leu-4 antibody. However, they are all unable to transport these proteins to their cell surface. Therefore, staining for CD3 antigen on NK cells, whether adult or fetal in origin, by using Leu-4 or rabbit anti-CD3ε, will produce cytoplasmic rather than membrane-localized signals. Hence, it is very important to discriminate membrane from cytoplasmic staining of CD3, before any conclusion on NK lineage is drawn.

This difficulty of identifying tumor cells in pleomorphic T-cell lymphomas with heterogeneous CD3ε staining has also been described in nonnasal tumors. Although we also favor the NK-cell origin of the majority of nasal lymphomas seen in Oriental populations, we do not agree that this conclusion can be based on the pattern of CD3 positivity when cytoplasmic and membrane type staining is not distinguished or when dual labeling methods are not used to assist in the identification of tumor and reactive cell
We recently found that nasal lymphomas could be classified into three distinct groups in terms of T-cell receptor (TCR) gene rearrangement and immunohistochemical staining with two markers of CD3 and CD56 and hypothesized that the cellular origin of these groups might correspond to adult natural killer (NK) cells, fetal NK cells, and T cells.\(^1\) In Tao et al.’s letter, they raised questions on our hypothesis mainly based on the uncertainty of the morphologic and immunohistochemical identification of neoplastic cells.

As has been pointed out in their letter and a previous publication,\(^2\) when lymphomas have polymorphous features it is difficult to distinguish reactive lymphocytes from neoplastic cells by morphologic features and single immunohistochemical staining on frozen sections. In all 9 cases we examined, polymorphous reticulosis was not found and neoplastic cells were easily recognized in their paraffin-embedded sections, although the identification of these cells on frozen sections was somewhat troublesome.

Ho et al reported previously that the nasal lymphomas of the "T"-cell phenotype were mostly TCR- (germline configuration of T-cell receptor) and that only 1 of 8 cases had rearranged TCR\(\gamma\) chain gene. They mentioned that nasal lymphoma with CD56- phenotype might possibly be of NK-cell lineage.\(^7\) Nasal lymphomas with polymorphic features were diagnosed as T-cell lymphoma when CD3+ cells were present. We had previously discussed this fact in our report.\(^1\) Because the expression of CD3 is not always limited to the cells of T-cell lineage but is also expressed rarely on NK-cell lineage, the rearrangement of TCR and Ig genes should be examined. Subsequently, we proposed the concept that, similar to B- and T-cell malignancies, NK-cell tumors could be classified by their differentiation stages. Surely our proposal fits into what Ho et al mentioned previously.\(^5\)

We agree with the claim of Tao et al on the difficulties of ascertaining whether CD3 positivity observed in CD56+ nasal lymphomas indicates tumor cells or reactive T cells. The best evidence to demonstrate the presence of CD3 and CD56 double-positive tumor cells would be obtained by a double-labeling study. As an alternative of this, we provide herewith the percentages of positivities of CD3 and CD56 cells (Table 1) that were not included in our previous paper. The percentages were calculated from the mean number of positive cells in three high power fields of sections stained by single immunohistochemistry. All positive cells in the tissues, such as endothelial cells, fibroblasts, and reactive lymphocytes, in addition to tumor cells, were included in the count. In at least 4 of 9 cases, the sum of TCR-CD56-CD2+ type with heterogeneous staining of CD3 antigen.

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5. Tao Q, Ho FCS, Loke SL, Srivastava G: Epstein-Barr virus is localized in the tumour cells of nasal lymphomas of NK, T or B cell type. Int J Cancer 60:315, 1995

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of the percentage of CD3+ and CD56+ cells was far greater than 100%, indicating the presence of double-positive cells. Although a single immunohistochemistry could not discriminate membranous and cytoplasmic positivity, we recognized that CD3 was mainly stained in the cytoplasm of lymphoma cells.

Because Ho et al showed clonal Epstein-Barr virus (EBV) integration in nasal lymphomas and that EBV+ cells represent a neoplastic population, we examined EBV in our nasal cases. Consistent with observations made previously by others, EBV was detected in all 9 cases and its clonality was verified by Southern blotting using terminal repeat sequence probe. Using the combination of immunohistochemical/in situ hybridization methods, Tao et al mentioned in their letter that all CD3+ cells were EBV+, although 20% to 50% of tissue cells were CD3+ in 2 cases of CD2+CD56+TCR- nasal lymphomas, and concluded that CD3+ cells were reactive T cells. We also examined the phenotype of EBV+ cells in our specimens using the same methods. For this purpose, oligo-probe-EBNA-region was used to detect EBV-RNA and anti-CD3ε (DAKO) and CD20 (L26) antibodies were used to detect phenotypic markers. As shown in our report, our nasal lymphoma cases included 3 CD3(Leu4)- cases (nos 3, 4, and 7) and 1 CD3ε+ case (no. 3) (Table 1). Virtually EBV+ cells were present in all cases. The proportion of EBV+ cells ranged from 30% to greater than 90% of the entire tissue cells and the sizes of EBV+ cells were mostly larger than that of reactive lymphocytes. In contrast to the findings of Tao et al in their letter, EBV and CD3ε double-positive cells were easily identified in the majority of our cases, except for the cases no. 3 and 4. On the other hand, consistent with the report of Mederios et al, only very few EBV+ cells were positive for CD20(L26).

In addition to these supplementary data, we recently had an experience with a 47-year-old man having nasal and mediastinal masses of lymphoblastic type lymphoma, of which tumor cells were double positive for CD3 and CD56, without rearrangement of TCRs. The nasal tumor tissue of this patient showed homogenous proliferation of tumor cells intermingled with a few macrophages so that typical polymorphic features of nasal lymphomas were not observed. Because CD3 and CD56 double-positive tumor cells were easily identified in such homogeneously proliferating tissues even in frozen section, this case would support the presence of CD3+CD56+ double-positive neoplastic cells (manuscript in preparation). In a related study, a Japanese group reported 2 cases of sinonasal "T"-cell lymphomas exhibiting both CD3 and CD56 antigens. Similarly, a Taiwan group reported a case with CD3+TCR-EBV+ nasal angiocentric lymphoma, although they did not mention CD56. To summarize, we provided supplementary results to demonstrate the presence of CD3 and CD56 double-positive cells at least in some nasal lymphomas. Furthermore, by double-labeling immunohistochemical/ISH methods, CD3ε+ cells were found among EBV+ cells in high frequency. On the basis of our findings and those of others, it is not surprising that nasal lymphomas of CD3+CD56+ neoplastic cells are in fact present. Because these cells do not exhibit TCR rearrangement, direct proof of the clonality of CD3+CD56+EBV+ should await the establishment of such cell lines from nasal lymphomas.

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Table 1. Positivity of CD3 and CD56

<table>
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<th>Patient No.</th>
<th>Age/Sex</th>
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<th>CD3(Leu4)+ (%)</th>
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Fig 1. Double-labeling immunohistochemical/in situ hybridization. EBV+ cells show dark brown-colored products in their nuclei and CD3ε+ cells show a red-colored reaction in their cytoplasm. (a) Patient no. 6 with CD3(Leu4)+CD3ε+; (b) patient no. 7 with CD3(Leu4)+CD3ε+.


TCR-CD56+CD2+ nasal lymphomas with membrane-localized CD3 positivity: are the CD3+ cells neoplastic or reactive? [letter; comment] [see comments]

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