CORRESPONDENCE

Presence of t(2;5) in Primary CD30+ Cutaneous Lymphoproliferative Disorders

To the Editor:

We read with interest the paper of DeCoteau et al[1] reporting the lack of t(2;5) chromosomal translocation in cutaneous CD30+ lymphoproliferative disorders by comparison with its presence in nodal anaplastic large-cell lymphoma (ALCL). The search for t(2;5)-encoded NPM-ALK transcripts was performed in 10 cases of lymphomatoid papulosis (LyP) and in only 2 cases of primary cutaneous CD30+ ALCL.[1]

These results are in contradiction with the report of Lopategui et al[2] on t(2;5) detection in 2 cases of primary cutaneous ALCL and with our recent report on the detection of NPM-ALK transcripts in 5 of 13 CD30+ primary cutaneous lymphomas and in 3 of 11 LyP.[3]

Such discrepancies have been observed for series dealing with the presence or absence of t(2;5) in Hodgkin's disease.[4-6] Several pitfalls, including cross-over contamination or polymerase chain reaction (PCR) assay low efficiency, have been raised in *Blood*.[7,8] While remaining aware of these problems, our study was first conducted in parallel in two independent laboratories using either a nested or an heminested PCR assay with sensitivities of t(2;5) detection at dilutions of 10^{-5} to 10^{-6}.[3,9] Such a sensitivity has not been tested in the report of DeCoteau et al[1] and amplification of a housekeeping gene mRNA is not a satisfying control for reverse transcription-PCR efficiency. Moreover, several groups have found nested amplification more reliable than standard PCR for the detection of NPM-ALK transcripts in nodal or systemic ALCL.[9-11] With extensive controls for cross-contamination, our report emphasizes the need of nested amplification when studying skin specimens in which lymphomatous cells are diluted in both normal and inflammatory cells. This is especially relevant for the study of LyP and would explain the negative results for t(2;5) detection in the series of Wellman et al[6] and DeCoteau et al.[1]

Moreover, the presence of t(2;5)-bearing cells in primary cutaneous CD30+ ALCL was confirmed both by in situ hybridization for the specific detection of NPM-ALK transcripts and immunodetection of p80 NPM-ALK.[3] Although DeCoteau et al[1] have used anti-p80 antibody at a dilution of 1:10,000, we used the antibody at lower dilutions ranging from 1:50 to 1:200, as reported previously[9,12] and as recommended by the manufacturer. Moreover, we have immunostained several sections for the detection of rare p80-positive cells in 2 cases of LyP.

Although DeCoteau et al[1] did not provide staging data, all patients included in our study fulfilled the criteria of Beljaard's et al[13] for the definition of primary cutaneous lymphoma. Therefore, our report of common molecular alteration in CD30+ systemic and cutaneous lymphomas does not support the claim for biologic differences between the two entities. Interestingly, t(2;5) has been found in a subset of nodal lymphomas sharing clinical features with cutaneous ALCL, ie, better prognosis and age of patients.[10,14] Finally, t(2;5) translocation with a protein tyrosine kinase (ALK) overexpression is the first oncogenic alteration shared by subsets of cutaneous ALCL and LyP that would reinforce the theory on a continuous spectrum between the two diseases.[15]

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REFERENCES

1. DeCoteau J, Butmarc JR, Kinney M, Kadin M: The t(2;5) chromosomal translocation is not a common feature of primary cutaneous CD30+ lymphoproliferative disorders: Comparison with anaplastic large-cell lymphoma of nodal origin. Blood 87:3437, 1996
2. Lopategui J, Sun L, Chan J, Gaffey M, Frierson H, Glackin C, Weiss L: Low frequency association of the t(2;5)(p23;q35) chromosomal translocation with CD30+ lymphomas from American and Asian patients. Am J Pathol 146:323, 1995
3. Beylot-Barry M, Lamant L, Vergier B, DeMuret A, Fraitag S, Delord B, Dubus P, Vaillant L, Mc Grogan G, Delaunay M, Beylot C, DeMascarel A, Delsol G, Merlio JP: Detection of t(2;5)(p23;q35) translocation by reverse transcriptase polymerase chain reaction and in situ hybridization in CD-30-positive primary cutaneous lymphoma and lymphomatoid papulosis. Am J Pathol 149:483, 1996
4. Orscheschek K, Merz H, Hell J, Binder T, Bartels H, Feller AC: Large-cell anaplastic lymphoma-specific translocation (t[2;5][p23;q35]) in Hodgkin's disease: Indication of a common pathogenesis? Lancet 345:87, 1995
5. Weiss L, Lopategui J, Sun L, Kamel O, Koo C, Glackin C: Absence of the t(2;5) in Hodgkin's disease. Blood 85:2845, 1995
6. Wellmann A, Otsuki T, Vogelbruch M, Clark HM, Jaffe ES, Raffeld M: Analysis of the t(2;5)(p23;q35) translocation by reverse transcription-polymerase chain reaction in CD30+ anaplastic large-cell lymphomas, in other non-Hodgkin's lymphomas of T-cell phenotype, and in Hodgkin's disease. Blood 86:2321, 1995
7. Orscheschek K, Merz H, Hell J, Feller A: Presence of the t(2;5) in Hodgkin's disease. Blood 86:4383, 1995
8. Weiss L, Glackin C: Presence of the t(2;5) in Hodgkin's disease. Blood 86:4384, 1995
9. Lamant L, Meggetto F, Al Saati T, Brugières L, Bressac de Paillerets B, Dastugue N, Bernheim A, Rubié H, Terrier Lacombe M, Robert A, Brousset P, Rigal F, Schlaifer D, Shiota M, Mori S, Delsol G: High incidence of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease. Comparison of cytogenetic analysis, RT-PCR and p80 immunostaining. Blood 87:284, 1996
10. Downing J, Shurtleff S, Zielenska M, Curcio-Brint A, Behm F, Head D, Sandlung J, Weisenburger D, Kossakowska A, Thorner P, Lorenzana A, Ladanyi M, Morris S: Molecular detection of the (2;5) translocation of non-Hodgkin's lymphoma by reverse transcriptase-polymerase chain reaction. Blood 85:3416, 1995
11. Yee H, Ponzoni M, Merson A, Goldstein M, Scarpa A, Chilosi M, Menestrina F, Pittaluga S, De Wolf-Peeters C, Shiota M, Mori S, Frizzera G, Inghirami G: Molecular characterization of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma (Ki-1) and Hodgkin's disease. Blood 87:1081, 1996
12. Herbst H, Anagnostopoulos J, Heinze B, Dürkop H, Hummel M, Stein H: ALK gene products in anaplastic large cell lymphomas and Hodgkin's disease. Blood 86:1694, 1995

Blood, Vol 88, No 8 (October 15), 1996: pp 3240-3249

13. Beljaards RC, Kaudewitz P, Berti E, Gianotti R, Neumann C, Rosso R, Paulli M, Meijer CJLM, Willemze R: Primary Cutaneous CD-30+ large cell lymphoma: Definition of a new type of cutaneous lymphoma with a favorable prognosis. Cancer 71:2097, 1993

14. Shiota M, Nakamura S, Ichinohasama R, Abe M, Akagi T, Takeshita M, Mori N, Fujimoto J, Miyauchi J, Mikata A, Nanba K, Takami T, Yamabe H, Takano Y, Izumo T, Nagatani T, Mohri N, Nasu K, Satoh H, Katano H, Fujimoto J, Yamamoto T, Mori S: Anaplastic large cell lymphomas expressing the novel chimeric protein p80 NPM/ALK: A distinct clinicopathologic entity. Blood 86:1954, 1995

15. Kadin M: Lymphomatoid papulosis and associated lymphomas. Arch Dermatol 129:351, 1993

Response

Beylot-Barry et al raise some theoretical concerns about the validity of our negative findings in CD30$^+$ cutaneous lymphoproliferative disorders (CD30$^+$ LPDs) and our conclusion that these entities likely evolve from different molecular pathways than primary CD30$^+$ ALCL of nodal origin.

Firstly, we take exception with their statement that "amplification of a housekeeping gene mRNA is not a satisfactory control for reverse transcription-PCR efficiency." Rather, we believe that amplification of a housekeeping gene mRNA represents the standard internal positive control to show the presence of amplifiable RNA. The question raised by Beylot-Barry et al relates more to the need to perform a nested RT-PCR procedure to show the t(2;5). The references listed by Beylot-Barry et al to support their contention that nested RT-PCR is required do not address this question.[1-3] It is only in the report by Downing et al[1] that a reference to the requirement of nested PCR is found. In that report, Downing et al[1] state that only "in rare cases, a nested RT-PCR approach is required for the detection of the chimeric product." Furthermore, we feel that the high number of amplification cycles inherent in nested RT-PCR may produce results that are difficult to interpret, eg, the detection of *bcl-2*-J_H and *bcr-abl* fusion transcripts in peripheral blood mononuclear cells of normal individuals.[4,5]

We are unable to understand the concern of Beylot-Barry et al regarding the dilution of the p80 antibody used in our study. Rather than using a standardized antibody dilution, immunohistochemistry requires the proper titration of a given antibody lot against known positive and negative cases to optimize conditions. In our initial titration studies, we found that dilutions in the range of 1:50 to 1:200 produced unacceptable background staining. Our antibody dilution was optimized to avoid background staining and to specifically stain tumor cells in positive controls of nodal ALCL.

We also do not understand the basis of Beylot-Barry et al's statement that we did not provide adequate staging data. The criteria of Beljaards et al[6] are useful to help distinguish primary cutaneous CD30$^+$ LPDs from CD30$^+$ ALCLs of nodal origin that secondarily involve cutaneous sites. We detected the t(2;5) in 5 of 5 cases of nodal CD30$^+$ ALCL that involved the skin. The inadvertent inclusion of nodal lymphomas in our group of primary cutaneous CD30$^+$ ALCL, as implied by Beylot-Barry et al, would have biased our results in favor of detection of the t(2;5). Again, we did not detect the t(2;5) in any of our cases of primary cutaneous CD30$^+$ LPDs, including 3 cases of CD30$^+$ ALCL and 1 borderline case, which is also in agreement with the findings of others.[7]

Furthermore, we question the significance of Beylot-Barry et al's detection of the t(2;5) in a minority of primary cutaneous CD30$^+$ LPDs using multiple rounds of PCR amplification. In keeping with our comments above, we believe that some PCR protocols are prone to the detection of fusion transcripts with oncogenic potential in bystander cells. These considerations, coupled with Beylot-Barry et al's statement that multiple tissue sections were required for the immunohistochemical detection of rare p80-positive cells in 2 of their 3 positive cases of lymphomatoid papulosis (LyP), adds to our concern about the biologic significance of their observations.

Finally, we have expanded our initial observation that the t(2;5) is not a feature of LyP. We have now evaluated a total of 19 cases of LyP and have not detected p80-positive atypical cells.

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REFERENCES

1. Downing JR, Shurtleff SA, Zielenska M, Curcio-Brint AM, Behm FG, Head DR, Sandlund JT, Weisenberger DD, Kossakowska AE, Thorner P, Lorenzana A, Ladanyi M, Morris S: Molecular detection of the (2;5) translocation of non-Hodgkin's lymphoma by reverse transcriptase polymerase chain reaction. Blood 85:3416, 1995

2. Lamant L, Meggetto F, Al Saati T, Brugieres L, Bressac de Paillerets B, Dastugue N, Bernheim A, Rubie H, Terrier Lacombe M, Robert A, Brousset P, Rigal F, Schlaifer D, Shiota M, Mori S, Delsol G: High incidence of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma and its lack of detection in Hodgkin's disease: Comparison of cytogenetic analysis, RT-PCR and p80 immunostaining. Blood 87:284, 1996

3. Yee H, Ponzoni M, Merson A, Goldstein M, Scarpa A, Chilosi M, Menestrina F, Pittaluga S, De Wolf-Peeters C, Shiota M, Mori S, Frizzera G, Inghirami G: Molecular characterization of the t(2;5)(p23;q35) translocation in anaplastic large cell lymphoma (Ki-1) and Hodgkin's disease. Blood 87:1081, 1996

4. Biernaux C, Loos M, Sels A, Huez G, Stryckmans P: Detection of major *bcr-abl* gene expression at very low levels in blood cells of some healthy individuals. Blood 86:3118, 1995

5. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJ, Schuuring E, Kluin PM: Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood 85:2528, 1995

6. Beljaards RC, Kaudewitz P, Berti E, Gianotti R, Neuman C, Rosso R, Paulli M, Meijer CJ, Willemze R: Primary cutaneous CD30-positive large cell lymphoma: Definition of a new type of cutaneous lymphoma with a favourable prognosis. Cancer 71:2097, 1993

7. Wellmann A, Otsuki T, Vogelbruch M, Clark HM, Jaffe E, Raffeld M: Analysis of the t(2;5)(p23;q35) by RT-PCR in CD30$^+$ anaplastic large cell lymphomas, in other non-Hodgkin's lymphomas of T-cell phenotype, and in Hodgkin's disease. Blood 86:2321, 1995