Malignant Lymphomas

The expression of TCR-γδ/CD3 complex in neoplastic γδ T-cell

We describe that the neoplastic γδ T-cells from patients with hepatosplenic γδ T-cell lymphoma have a lower expression of TCR-γδ/CD3 complex compared to their normal or reactive counterparts. Interestingly, with the use of an appropriate antibody association, this feature is easily and rapidly observed on flow cytometry graphics.

Haematologica 2006; 91:1717-1719
(*http://www.haematologica.org/journal/2006/12/1717.html*)

Hepatosplenic γδ T-cell lymphoma (HSγδTCL) is described as a neoplasm derived from non activated cytotoxic γδ T-cells and particularly from the Vδ1 subset.[1-5] γδ T-cells may be expanded in early immune responses to infections, auto-immune disorders and cancer surveillance especially in B-cell lymphomas,[6,7] and the study of immunological γδ T-cell pattern appears particularly helpful to avoid misdiagnosis with γδ T-cell neoplasm. Indeed reactive γδ T-cells are more often in an activated state, reflected, for example, by the expression of HLA-DR and CD25, in contrast to the non-activated profile of γδ T-cells from HSγδTCL. γδ T-cells are characterized by a higher expression of TCR-γδ/CD3 complex than are αβ T-cells, probably contributing to their sentinel role in immunity.[8]

We studied the levels of expression of the TCR-γδ/CD3 complex by neoplastic γδ T-cells from three patients with histologically proven HSγδ TCL and compared these with the levels in normal or reactive counterparts (specimens of spleen (n=10) and peripheral blood (n=16) from patients with acute viral syndrome, other T-cell lymphomas, B-cell lymphomas and without a specific hematologic diagnosis).

In all cases, the neoplastic γδ T-cells had the immunological profile commonly described in HSγδ TCL (Table 1).[1-5] This profile was consistent with their derivation from non-activated cytotoxic γδ T-cells, while the γδ T-cells from the control group had an activated cytotoxic profile (HLA-DR$^+$, CD25$^+$, perforin$^+$ and granzyme B7$^{+/-}$). The CD3 mean fluorescent intensity (MFI) ratio (γδ/αβ) was calculated for each patient and control. In the three patients, the median ratio was 0.7 [range 0.5 to 1.0] on T cells from spleen suspensions and 0.4 [range 0.1 to 0.9] on T cells from peripheral blood (Figure 1). These ratios were significantly lower than the ratios calculated for the control group 1.8 [range 1.6 to 2.0] on T cells from spleen suspensions and 2.0 [range 1.5 to 3.7] on T cells from peripheral blood. In addition, the MFI of the γδ-complex was lower in neoplastic γδ T-cells (MFI=1.1-median-) [range 1.0 to 1.2] than in reactive and/or non-tumoral γδ T-cells from spleen samples (MFI=3.3-median-) [range 2.0 to 6.0] or peripheral blood specimens : neoplastic γδ T-cells (MFI=1.6-median-)[range 1.3 to 2.0] and control group (MFI=4.7-median-)[range 3.2 to 7.9] (Figure 1). To our knowledge, this is the first report showing that neoplastic γδ T-cells are characterized by a lower expression of TCR-γδ/CD3 complex than their normal or reactive counterparts. The observation of abnormal expression of one or more pan-T antigens, such as loss or downregulation of CD3, is commonly used as a helpful criterion for the diagnosis of a T-cell lymphoproliferative disease.[9,10] However, one should keep in mind that atypical T-cell profiles can be observed in a reactive context. Among the control group, γδ T-cells were expanded in two specimens of spleen from cases of splenic marginal zone lymphoma and in ten peripheral blood samples but they had an activated profile and expression of the TCR γδ-/CD3 complex was within the range defined as normal in our study. In γδ T-cell expansion, the level of expression of the TCR-γδ/CD3 complex of the γδ T-cells therefore appears as a strong discriminator between neoplastic and reactive expansion. Importantly this immunological feature has the advantage of being easily and rapidly observed on flow cytometry graphics with a minimal set of antibodies (anti-TCR-αβ, TCR-γδ and CD3) (Figure 1) in contrast to the complete T-cell phenotype for which a broad panel of markers is essential.

All spleen specimens (patients and controls) expressed the vδ1 antigen in accordance with the concept that HSÁγδTCL derive from local lymphoid spleen tissue where vδ1 gene usage predominates.[5] The γδ T cells identified in the two cases of peripheral blood available for flow cytometric analysis expressed the vδ1 gene, whereas the γδ T-cells from peripheral blood from controls did not express the vδ1 antigen, as previously described.[1,5] This confirms the dissemination of HSγδTCL in the blood and indicates that the low expression of the TCR-γδ/CD3 complex on circulating γδ T-cells could be used as a marker of malignancy. Peripheral blood dissemination as well as bone marrow involvement is likely to be underestimated because of the difficulties in identifying neoplastic γδ T cells since these tumor cells present minimal cytologic atypia and secondly a normal lymphocytosis is generally reported at the initial examination. Low expression of the TCR-γδ/CD3 complex on γδ T cells in peripheral blood could, therefore, be used to detect circulating malignant cells and to orientate the diagnosis before

Table 1. Immunological profile of the three HSδγδTCL cases.

Case	Site	Ly	CD1a	CD2	CD3	CD4	CD5	CD7	CD8	CD16	CD25	CD45-RO	CD45-RA	CD56	CD57	HLA-DR	TCR-γδ	Vδ1	TIA-1	Perf	GrB7
1	S	0.3	–	+	+	–	–	+	–	+w	–	–	+	+	–	–	+	+	+	+w	–
2	S	12.0	–	+	+w	–	–	+	–	+	–	–	+	+	–	nd	+	+	+	–	–
	PB	12.0	–	+	+w	–	–	+	–	+	–	–	+	+	–	–	+	+	+	–	–
3	PB	5.0	–	–	+	–	–	+w	–	+w	–	–	+	–	+w	–	+	+	+	–	–

S: spleen; PB: peripheral blood; Ly: Lymphocytes (×10^9/L); Perf: perforine; GrB7: granzyme B7; w : weak. In case 1 no blood sample and in case 3 no initial spleen or blood samples were available for the flow cytometric immunological analysis; in case 3 the γδ T-cell profile was studied during the course of the disease.

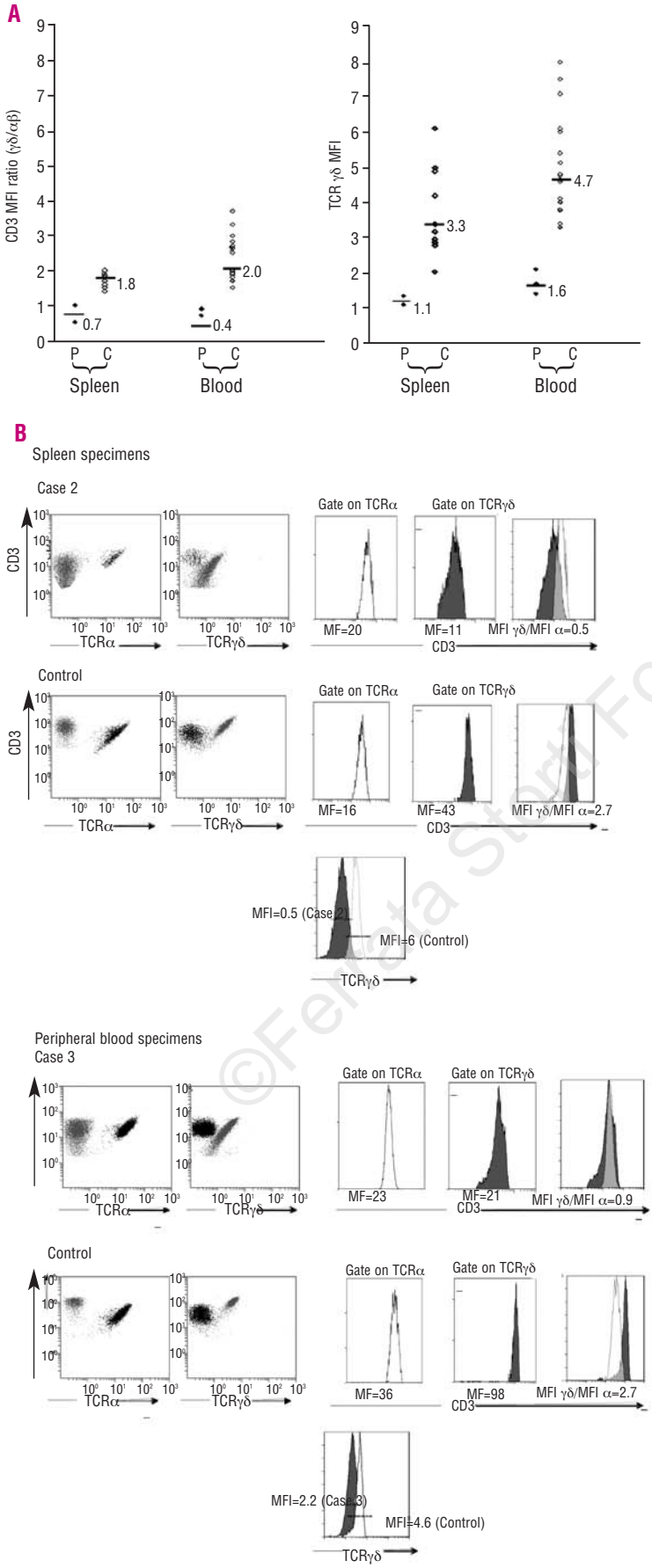

Figure 1. Low expression of TCR-γδ/CD3 complex on neoplastic T cells from a case of HSγδTCL **A.** CD3 MFI ratio (γδ/αβ) and TCR-γδ MFI from peripheral blood and spleen specimens in the group of patients (P) and the control group (C): the horizontal lines indicate the median. **B.** Flow cytometry graphics: direct three-color flow cytometric immunophenotyping was performed using TCR-γδ, TCR-αβ and CD3 antibodies, allowing selective gating on the γδ T-cell and αβ T-cell populations. The level of expression of CD3 on γδ T-cells and αβ T-cells from spleen and peripheral blood specimens was compared by calculating the ratio of CD3 MFI (γδ/αβ) in the group of patients and in the control group. The levels of expression of the γδ-complex on γδ T-cells from patients and T cells from controls were compared.

more invasive investigations in the case of suspected splenic lymphoma, especially when no B-cell clone has been found.

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Key words: γδ T-cells, HS γδTCL, TCR- γδ/CD3 complex, flow cytometry.

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