B-, T-, and NK-cell lineage involvement in *JAK2*^{V617F}-positive patients with idiopathic myelofibrosis

An acquired *JAK2*^{V617F} mutation has been found in myeloid cells from most patients with chronic idiopathic myelofibrosis (IM), but whether it occurs in a common myelo-lymphoid, rather than a myeloid-restricted, progenitor cell is still debated. Using a sensitive ARMS assay for the quantitative assessment of JAK2^{V617F} cDNA, we detected the mutation in purified B, T and NK cells from about half of 12 patients studied. These results indicate that involvement of the lymphoid lineage in IM may be more frequent than previously supposed.

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Patients with chronic myeloproliferative disorders (MPD) harbor a recurrent *JAK2*^{V617F} mutation[1] that has been consistently found in their granulocytes but not in control tissues. In cases in which T lymphocytes were analyzed, they resulted uniformly wild-type;[2,3] likewise, the mutation could not be found in CD19$^+$ cells purified from three homozygous cases of MPD using a direct sequencing approach.[4] These data would suggest that the cell target for the *JAK2*^{V617F} mutation is a myeloid-restricted progenitor. However, this idea is challenged by the finding that cells with the hematopoietic stem cell (HSC) phenotype (CD34$^+$, CD38$^-$, CD90$^+$, Lin$^-$) from patients with polycythemia vera (PV) actually harbored the mutation.[5] Furthermore, Ishii *et al.*[6] recently reported that B and T cells from, respectively, 2/8 and 1/8 PV patients were *JAK2*^{V617F} mutated. Interestingly, the mutation was also found in B, T, and NK cells of one patient with familial MPD.[7]

We analyzed 12 patients with IM, at variable times from diagnosis, searching for the presence of the *JAK2*^{V617F} mutation in their lymphoid cells. All patients gave informed consent to these investigations. Peripheral blood (PB) granulocytes were collected by density gradient centrifugation to ≥95% purity by visual inspection of cytosmears; CD3$^+$ and CD19$^+$ lymphocytes were obtained by direct immunomagnetic selection to a purity ≥98% by FACS re-analysis. In five patients, the purity of CD56$^+$/CD3$^-$ NK cells and CD3$^+$/CD56$^-$ T-lymphocytes obtained using an immunomagnetic depletion procedure was ≥93% and ≥95%, respectively. PB CD34$^+$ cells were purified to ≥97% by direct immunomagnetic selection (Miltenyi Biotec, Germany). The percentage of *JAK2*^{V617F} cDNA in these cell fractions was measured using an ARMS procedure.[8] Briefly, RNA was reverse transcribed and amplified using fluorochrome-labeled mutation-specific primers; amplicons were resolved by capillary electrophoresis, and the ratio of *JAK2*^{V617F} to *JAK2*^{total} (*JAK2*^{V617F}+JAK2^{WT}) cDNA was calculated.[8]

We observed a significant, although heterogeneous, pattern of lymphoid cell involvement in IM patients (Table 1). In particular, the *JAK2*^{V617F} mutation was detected in the CD19$^+$ cells of 7/12 patients (58%), in the CD3$^+$ cells of 5/12 (42%), and in the CD56$^+$ NK cells of all the five cases in which they were evaluated. Remarkably, the *JAK2*^{V617F} ratio measured in these highly-purified lymphoid cell preparations was high enough, when compared to the corresponding value in granulocytes, to exclude the possibility that the presence of the mutation may have been due to a few contaminating granulocytes.

Table 1. The *JAK2*^{V617F} mutant allele ratio in different hematopoietic cell fractions purified from the peripheral blood of patients with idiopathis myelofibrosis.

	JAK2^{V617F}/JAK2total ratio (%)					
Pat. #	*CD34*$^-$	*GN*	*CD19*$^+$	*CD3*$^+$	*CD3*$^+$/*CD56*$^-$	*CD56*$^-$/*CD3*$^-$
1	100	100	0	0	0	ND
2	13	15	0	0	0	ND
3	89	60	43	28	20	30
4	41	50	44	37	ND	ND
5	42	25	22	15	ND	ND
6	16	24	0	0	0	ND
7	73	96	0	0	0	ND
8	29	26	30	0	0	ND
9	100	100	18	0	0	35
10	33	48	25	ND	20	24
11	100	100	100	ND	100	100
12	13	40	0	0	0	19

ND: not done; GN: granulocytes; "0" means wild-type JAK2: CD3$^+$ cell fractions indicate T-lymphocytes positively selected using anti-CD3 antibodies; since these fractions might contain CD3$^+$/CD56$^-$ NK cells, in some patients CD3$^+$/CD56$^-$ T cells were obtained by a negative immunoselection procedure.

The presence of the *JAK2*^{V617F} mutation in lymphoid cells was not obviously associated with the highest mutational load in myeloid cells; for example, patient #1 had a *JAK2*^{V617F} ratio of 100% in granulocytes, while both CD9$^+$ and CD3$^+$ were wild-type, and conversely patient #8, in whom a *JAK2*^{V617F} ratio of 26% was found in granulocytes, had a ratio of 30% in CD19$^+$ cells and wild-type CD3$^+$. Patient #11, who had a 6-year long history of the disease, was particularly interesting and presented a 100% ratio in all the different cell fractions examined.

We also found that CD34$^+$ cells harbored the *JAK2*^{V617F} mutation at ratio values roughly similar to those in granulocytes. Although immunomagnetically selected CD34$^+$ cells contain both CD38$^+$ and CD38$^-$ cells, and thus do not truly reflect a HSC phenotype, nonetheless this observation suggests that the target cell in IM (and PV)[6] may be a common myelo-lymphoid progenitor,[9] and indirectly makes it unlikely that the phenotypic pleiotropy of *JAK2*^{V617F} mutated MPD patients results from different cellular targets.

Overall, the current data are in line with those recently reported by Delhommeau *et al.*[9] in ten IM patients. These authors found the *JAK2*^{V617F} mutation in B-cells of 60% of the patients, in 25% in case of T-cells, and 63% of NK cells. The variable frequency of lymphoid cells involved in the *JAK2*^{V617F} mutant clone might be also explained by the long lifespan of these cells, especially T-lymphocytes, most of which actually pre-existed the event(s) leading to the acquisition of the *JAK2*^{V617F} mutation. Consistently, *JAK2*^{V617F} mutant T cells were found in all IM (and PV) patients evaluated using the fetal thymus organ culture (FTOC) assay.[9]

Larger studies are clearly needed to obtain a consistent figure of the incidence of *JAK2*^{V617F} mutation in lymphoid cells, but from these data it appears to be not sporadic; specifically, the low-sensitivity direct sequencing approach employed in the first studies might have been the reason for the under-estimation of this phenomenon.[2-4] The consequences, if any, of the involvement of B cells, T cells and NK cells by the *JAK2*^{V617F} mutation are

totally unclear so far, but one is tempted to speculate that it might underlie some of the immunologic abnormalities manifested by a significant proportion of IM patients.[10]

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