Chronic Myeloproliferative Disorders

Immunophenotypic evaluation of circulating T-cell clones in hypereosinophilic syndromes with or without abnormal CD3 and CD4 lymphocytes

T-cell immunophenotype and status of the Vβ repertoire were evaluated by flow cytometry in patients with hypereosinophilic syndromes (HES). Abnormal T-cell phenotype and/or clonality were found in 6/12 cases ($CD3^-$ and $CD3^+$ clones; minor T-cell clones). Immunophenotyping evaluation may facilitate the subclassification of HES and assist therapeutic interventions and monitoring.

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Primary hypereosinophilic syndrome (HES) is a protean entity associated with marked eosinophilia in the absence of recognizable etiopathogenetic factors.[1] Other eosinophil-rich disorders include chronic eosinophilic leukemia (CEL), eosinophil-rich myelodysplasia (MDS-eo),[2,3] and chronic idiopathic eosinophilia (CIE), which resembles HES but causes no clinical harm. The treatment of these syndromes is difficult. Before the introduction of imatinib mesylate, adrenal corticosteroids were regarded as a therapeutic cornerstone. Because their activity is negligible in myeloid proliferations, where they actually stimulate rather than inhibit the cell growth, it is intriguing to consider how corticosteroids can be drastically effective in syndromes whose cell lineage affiliation is clearly in the myeloid rather than the lymphoid compartment.

To understand this paradox better, we need to focus on the array of T-cell abnormalities reported in HES. Abnormal T-cell clones (TCC), often with a $CD3^-$ $CD4^+$ phenotype, have been described.[4-6] TCC would promote eosinophilia through cytokine release, principally interleukin-5.[7] Less frequently, abnormal T cells bear a $CD3^+$ $CD4^-$ $CD8^{-/-}$ phenotype,[8] but exact data on frequency and types of TCC in HES, CEL and CIE are lacking. Altogether, the whole data enforce the view that therapy, in selected circumstances, should target an underlying TCC, also explaining the corticosteroid response and calling for the evaluation of other T-cytotoxic drugs.

We studied 12 patients with a diagnosis of HES, CEL/MDS-eo or CIE.[1,2] The degree of eosinophilia was defined as mild (>0.35-1.5×10^9/L), moderate (>1.5-5×10^9/L) or severe (>5×10^9/L). TCC were identified cytofluorometrically employing a validated technique that recognizes 24 different T-cell receptor (TCR) clonal rearrangements of the Vβ domains.[9,10] Immunophenotype was evaluated using fluorochrome-conjugated monoclonal antibodies (to CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56, CD57). For TCC analysis, the IO Test Beta Mark eight-tube kit was used (Beckman Coulter Inc., Instrumentation Laboratory, Milan, Italy). Each kit tube contains 4 fluorochrome-conjugated monoclonal antibodies: CD3-PerCP plus three different Vβ antibodies directly conjugated with FITC, PE, and FITC/PE, respectively. The normal upper limit of reactivity for each Vβ antibody has already been reported.[9,10]

The characteristics of the patients are shown in Table 1. Four patients had HES, two had MDS-eo, one had CEL (progressing to acute myelogenous leukemia), and 5 had CIE. Levels of blood eosinophilia varied either spontaneously (CIE) or following therapeutic intervention at different stages of the disease. At study, an absolute lymphocytosis was seen only in one HES patient. As concerns cytometry study results (Table 1), six patients (50%) had an abnormal T-cell phenotype (TCP) and/or Vβ antibody reactivity to suggest a TCC. The latter was documented in two patients with HES, one with CEL and three with CIE, including the single HES case with absolute lymphocytosis >4×10^9/L. Two cases exhibited an abnormal TCP with concurrent TCC (case 3, $CD3^-$ $CD4^+$; case 9, $CD3^+$ $CD4^-$ $CD8^-$); interestingly in case 3 the clonal Vβ domain was detected in $CD3^-$ $CD4^+$ cells, to suggest a likely cytoplasmic expression of the molecule. Three other patients bore a TCC with no apparent surface marker abnormality (#2, 5 and 8), and in a last case with an abnormal TCP (#9, with $CD3^-$ $CD4^-$ $CD8^-$ cells) there was no evidence of an associated TCC. Figure 1 illustrates representative flow cytometry tests from two of these patients.

In summary, T-cell alterations in hypereosinophilic syndromes may be frequent and do not necessarily correlate with blood lymphocytosis. In addition the occurrence of an abnormal TCP may not always indicate an underlying TCC

Table 1. Patients with hypereosinophilic syndromes: cytofluorimetric evaluation of T-cell phenotype (TCP) and clonality (TCC).

No.	*Age/Sex*	*DEo*[1]	*Diagnosis (symptoms)*	*Treatment*	*EoD/S*[2]	*LyD/S*[3]	*TCP*	*TCC*
1	37/f	1.4	HES (skin)	P	0.9/0.9	3.0/2.4	N	N
2	61/m	7	HES (skin, gut)	P, MP, HU, IFN	16.1/1.1	2.6/2.0	N	Vβ2 (17%)
3	42/m	1.2	HES (heart, spleen)	P, IFN	40.1/0.7	6.0/6.3	$CD3^-4^+$ (20%)	Vβ2 (15%)
4	60/m	8	HES (skin, spleen)	P, HU	15.2/3.7	2.0/1.2	N	N
5	50/m	10	CEL (AML)	P, HU, CC, MP, IFN	11.4/1.2	6.3/0.3	N	Vβ2 (17%)
6	50/m	3	MDS-eo with t(1;5)	IM	2.6/2.7	2.2/2.2	N	N
7	19/m	3.7	MDS-eo with t(4;8)	HU, IFN	2.9/1.6	1.6/2.3	N	N
8	78/f	2.0	CIE	–	14.2/4.9	3.7/1.3	N	Vβ211 (13.5%)
9	62/f	1	CIE	–	4.9/4.9	1.3/1.3	$CD3^+4^-8^-$ (57%)	Vβ213.6 (62%)
10	81/f	1.5	CIE	–	1.5/1.9	na/0.8	N	N
11	57/m	1.5	CIE	–	1.9/1.2	1.9/1.9	$CD3^-$ (40%)	N
12	27/m	6	CIE	–	1.7/1.1	2.7 /2.1	N	N

[1]DEo: duration of hypereosinophilic syndrome (years); [2]EoD/S: blood eosinophilia (×10^9/L) at time of diagnosis/study; [4]LyD/S: lymphocyte count (×10^9/L) at time of diagnosis/study; na, not available; TCP/TCC: abnormal T-cell phenotype/T-cell clone (N means normal, following comparisons with values obtained from normal controls; abnormal findings are detailed to indicate phenotype and/or clonality of the abnormal T-cells (per cent values in brackets refer to the proportion of positive T-cells within total T-cells); P, prednisone or other corticosteroid; MP, 6-mercaptopurine; HU, hydroxyurea; IFN, α-interferon; CC, combination chemotherapy; IM, imatinib mesylate.

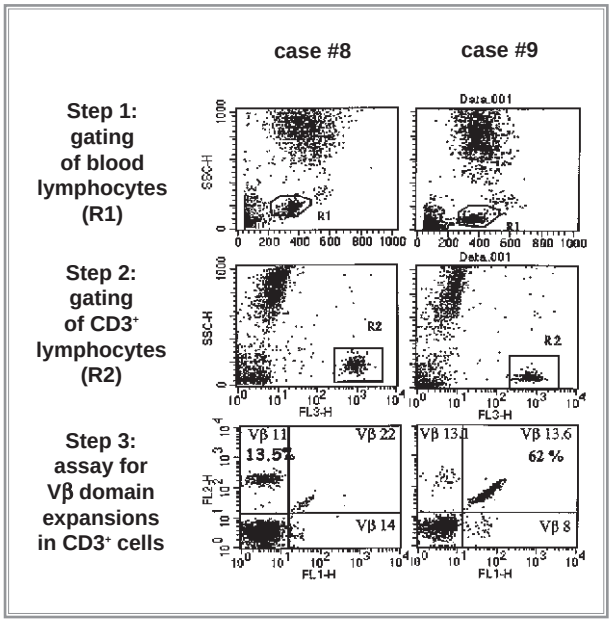

Figure 1. Three-step flow cytometric analysis of Vβ repertoire in CD3⁺ cells: (1) gating of blood lymphocytes; (2) gating of CD3⁺ lymphocytes within total cells; (3) analysis of Vβ antibody reactivity in CD3⁺ cells from cases #9 (CD3⁺ CD4⁻ CD8⁻ TCC, 57-62% of all CD3⁺ T-cells) and #8 (minor TCC,13.5%, without TCP abnormalities).

and viceversa. In fact minor TCCs were identified in cases with normal TCP. Thus, normal CD3⁺ CD4⁺ (or CD8⁺) subsets may harbor a clonal cell population just like the more atypical CD3⁻ CD4⁺ or CD3⁺ CD4⁻ CD8⁻ lymphocytes can do. Whether this reflects oligoclonality of CD3⁺ CD8⁺ (or CD4⁺) cells, as sometimes reported in elderly healthy controls,[10] or is the very early phase of an eosinophilia-associated T-cell disorder has yet to be determined. Further monitoring of these patients is indicated. In the single case with expanded, Vβ-unreactive CD3- cells, Southern blotting or PCR may be required to rule out clonality and eventually confirm a concurrent CD3- NK cell increase (about 40%, *data not shown*).

The diagnostic technique described by Langerak *et al.*[9] appears sufficiently sensitive for an accurate assessment of individual amounts of circulating TCC in patients with hypereosinophilic disorders. Although the formal proof of a clonal T-cell disease rests on molecular biology techniques and about 30% of known Vβ rearrangements are not covered by this method, flow cytometric analysis allows fast screening and could be useful, in patients with associated TCC, in the monitoring of treatment response and disease progression.

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Chronic Myeloproliferative Disorders

A further case of myeloproliferative syndrome with reciprocal translocation (8;13)(p11;q12)

The 8p11 myeloproliferative syndrome is an aggressive stem cell disorder characterized by eosinophilia, lymphadenopathy and rapid progression to acute leukemia, caused by FGFR1 fusion proteins resulting from translocations at chromosome band 8p11. We report a new case initially responsive to aggressive chemotherapy, who progressed to acute leukemia despite allogeneic stem cell transplantation.

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Several subtypes of eosinophilic leukemia have been recently identified. These rare disorders are associated with specific cytogenetic and molecular genetic abnormalities.[1] A chronic eosinophilic leukemia which often evolves into T-lymphoblastic lymphoma or acute myeloid leukaemia (AML) is characterized by translocations with a chromosome 8p11-12 breakpoint and is, therefore, known as 8p11 myeloproliferative syndrome (EMS).[2] This disorder is extremely aggressive: only allogeneic stem cell transplantation seems effective in eradicating the neoplastic clone. We report a patient with EMS who initially responded to aggressive chemotherapy, but soon evolved to AML despite allogeneic peripheral blood stem cell transplantation.