## Hypereosinophilia with abnormal t cells, trisomy 7 and elevated tarc serum level

**The idiopathic hypereosinophilic syndrome (HES) is a rare heterogeneous disorder, characterized by persistent blood eosinophilia with possible organ involvement. We describe here the case of a 20-year-old atopic male presenting chronic hypereosinophilia and eczema since childhood. Biological findings included hypereosinophilia (9.5x10⁹/L), hyperlymphocytosis (10.9x10⁹/L), polyclonal hypergammaglobulinemia and elevated IgE serum level. Flow cytometric analysis of blood lymphoid cells showed a population of $CD2^{+}CD3^{-}CD4^{+}TCR\alpha\beta^{-}TCR\gamma\delta^{-}$ lymphocytes. These cells displayed a Th0/Th2 cytokine profile, and a clonal TCR rearrangement pattern. A high serum TARC level was observed. Karyotype studies on blood stimulated culture or lymph nodes revealed a cellular hyperdiploïd clone 47, XY, +7. To our knowledge, this chromosomal aberration has never been reported in such a case.**

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*Introduction*

The etiology of persistent hypereosinophilia includes parasitic or allergic disorders, solid tumors and hematologic malignancies.[1-4] Recent data on idiopathic hypereosinophilic syndrome (HES) point to regulation defects in T lymphocytes.[5,6] Clonal Th2 lymphocytes appear to be implicated in some cases of HES through secretion of cytokines such as interleukin IL-4, IL-5, and IL-13.[6-11] Indeed, Th2 cytokines are known to contribute to recruitment, activation and survival of eosinophils.[12] The lymphocytic variant of HES is defined as a distinct entity in which eosinophilia is likely to be caused by overproduction of IL-5 by a Th2-like lymphocyte subset.[11] Several investigators have described lymphoma progression in this setting.[6,11] Although chromosomal abnormalities in HES are considered to be indicative of eosinophilic leukemia,[1,3,4] 3 patients with the lymphocytic variant were reported to present aberrant karyotypes : 46, XY, -16, +der[16,9] and partial deletions of chromosomes 6 and 10.[11] Here, we report the first case of combined hypereosinophilia and hyperlymphocytosis (CD3-CD4+) associated with trisomy.

*Methods*

*Cytometric analysis.* Flow cytometric analysis of lymphocytes was performed either on EDTA-treated blood or on PBMC with MoAbs against CD2, CD4, CD11b, CD62L, CD3, CD27, CD30, CD5, CD8, and CD16 from Beckman Coulter and CD19, CD25, CD28, CD45RA, CD45RO, CD95, CD95L, TCRαβ, TCRγδ from BD Biosciences. For measurement of intra-cytoplasmic cytokine expression, PBMC were prepared according to a previously described protocol.[11] Intra-cytoplasmic CD3 and TCR expression was evaluated by flow cytometry after paraformaldehyde fixation and saponin permeabilization of PBMC.

*PCR analysis of the T-cell receptor (TCR) genes and IgH genes.* To determine lymphocyte clonality, a PCR amplification based on the rearrangement of TCR genes and IgH genes was used.[13] Blood was collected on EDTA; lymphocytes were obtained after Ficoll and cytometric selection. Extraction of DNA was performed using the Qiamp technique (Quiagen). Oligonucleotides provided by Eurogentec (Belgium) were FITC-labeled. The fluorescent PCR product was analyzed by electrophoresis on a 377ABIPRISM.® Seven PCR reactions were performed systematically ( for the gamma chain of the TCR : VγIJIJ2, VγIJP1/2, Vγ9JIJ2; for the delta chain of the TCR : Vδ1Jδ1, Vδ2Dδ3 and for IgH : FR1-JHc, FR3-MC4). To assure quality of the DNA, a control Mut S Homolog 2 (MSH2) PCR was performed. MSH2 is a gene involved in the postreplicative DNA mismatch repair system to maintain genomic integrity and is expressed ubiquitously.

| PCR | Primer | Sequence |
|---|---|---|
| VγIJIJ2 | VγI Sense | 5'-CTA-CAC-CAG-GAG-GGG-AAG-G-3' |
| | J1J2 Antisense | 5'-ATT-CTT-CCG-ATA-CTT-ACC-TGT-GA-3' |
| VγIJP1/2 | VγI Sense | 5'-CTA-CAC-CAG-GAG-GGG-AAG-G-3' |
| | JP1/2 Antisense | 5'-TTA-CCA-GGT-GAA-GTT-ACT-ATG-AGC-3' |
| Vγ9JIJ2 | Vγ9 Sense | 5'-GAA-AGG-AAT-CTG-GCA-TTC-CG-3' |
| | J1J2 Antisense | 5'-ATT-CTT-CCG-ATA-CTT-ACC-TGT-GA-3' |
| Vδ1Jδ1 | Vδ1 Sense | 5'-CGC-CTT-AAC-CAT-TTC-AGC-CTT-ACA-GCT-AGA-3' |
| | Jδ1 Antisense | 5'-CCT-TAA-CCT-TAA-ACT-TCA-GAT-AAA-TAA-ATG-AGT-TAC-3' |
| Vδ2Dδ3 | Vδ2 Sense | 5'-TGG-CCC-TGG-TTT-CAA-AGA-CAA-TTT-CCA-3' |
| | Dδ3 Antisense | 5'-TGA-GGA-TAT-CCC-AGG-GAA-ATG-GCA-CTT-3' |
| FR1-JHc | FR1 Sense | 5'-AGG-TGC-AGC-TG(G/C)-(A/T)G(G/C)-AGT-C(G/A/T)G-G-3' |
| | JHc Antisense | 5'-ACC-TGA-GGA-GAC-GGT-GAC-C(A/G)(G/T)-(G/T)GT-3' |
| FR3-MC4 | FR3 Sense | 5'-ACA-CGG-C(TC)(GC)-TGT-ATT-ACT-G-3' |
| | MC4 Antisense | 5'-ACC-TGA-GGA-GAC-GGT-GAC-C-3' |
| MSH2 | Sense | 5'-CGC-GAT-TAA-TCA-TCA-GTG-3' |
| | Antisense | 5'-GGA-CAG-AGA-CAT-ACA-TTT-CTA-TC-3' |

*Karyotype analysis.* PBMC were stimulated by phorbol 12-myristate 13-acetate 5 ng/mL plus ionomycin 500 ng/mL (Sigma) for 48 hours and metaphases were analyzed using standard banding techniques.

*Measurement of serum thymus and activation-regulated chemokine (TARC) levels.* TARC levels were determined using a standard ELISA kit from R&D Systems Europe.

*Measurement of cytokine levels.* IL-2, IL-4, IL-5, interferonγ, TNFα, IL-10 serum levels were determined by using the human Th1/Th2 cytokines CBA kit from BD Biosciences. IL-5 and GM-CSF were measured on culture supernatants using ELISA kits from Immunotech. The sensitivity of the ELISA kit was 1pg/mL for IL-5 and 7 pg/mL for GM-CSF.

*Result and discussion*

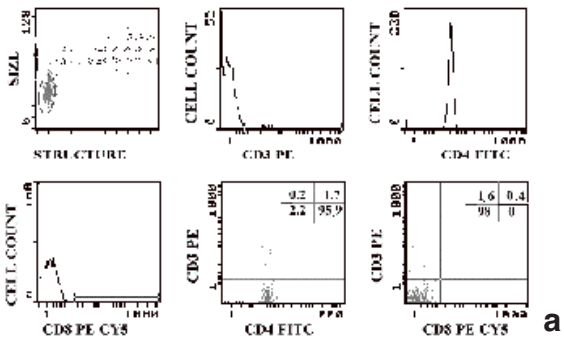
*Case report.* A 20-year-old atopic man with a history of eczema since childhood and chronic hypereosinophilia was admitted (Hematology Dept. Beauvais Hospital, France) because of pain in the lower back and finger joints. He complained of generalized pruritus and episodic angioedema of the forearms and ankles. Clinical examination revealed typical skin lesions due to eczema and many enlarged superficial lymph nodes. The patient was otherwise healthy. Biological findings included

unexplained hyperlymphocytosis (10.9x10⁹/L), hypereosinophilia (9.5x10⁹/L), polyclonal hypergammaglobulinemia and high serum IgE level. Bone marrow aspiration revealed infiltration of eosinophils, abundant eosinophil precursors and lymphoid cells. All these findings did not seem to fit with a single atopic origin. Histologic examination of lymph node showed reactive lymphoid hyperplasia with marked infiltration of mature eosinophils.

*Blood lymphocyte immunophenotypic analysis.* Only 2 to 3 % of peripheral blood lymphocytes were $CD3^+$ $CD4^+$ $CD8^-$ whereas 95.7 % of total lymphocytes (reaching $29.4x10^9$ cells /L) were $CD3^-$ $CD4^+$ $CD8^-$ (Figure 1A). These lymphoid cells were $CD7^-$, $CD16^-$, $CD19^-$ and showed a mature memory phenotype ($CD45RO^+$, $CD62L^+$). They did not express surface ab or gd TCR, despite expression of CD4, CD28 and of the pan-T-cell markers CD2 and CD5. Intracellular staining for TCR ab or gd was negative and a low expression of CD3 was observed in the cells. Activation marker HLA DR was expressed at high levels whereas CD25 was absent (Figure 1B). $CD3^-CD4^+$ lymphocytes can be detected in the normal thymus where they differentiate from $CD3^-$ $CD4^-$ $CD8^-$ precursors and subsequently mature into $CD3^+$ $TCR^+$ $CD4^+$ or $CD3^+$ $TCR^+$ $CD8^+$.[14] Circulating $CD3^-$ $CD4^+$ cells have been found in some patients with hematologic diseases,[1] and some viral infections prevent cell surface expression of the T-cell receptor, *in vitro*.[15] Our patient had negative HIV and HTLV1 serology. One group has suggested that disrupted Fas-mediated apoptosis could be responsible for expansion of aberrant T cells lacking CD95 in patients with HES,[6] but in the studied patient, lymphocytes were $CD95^+$. A previous study has shown that proliferation of $CD3^-$ $CD4^+$ cells in response to dendritic cells *in vitro* was dependent upon engagement of CD2 and CD28 and on an IL-2 / IL-2R autocrine loop.[5]

*TARC serum level.* Since it has been recently proposed that the chemokine TARC could play a role in Th2 lymphocyte recruitment (16-18), we measured the serum TARC level and found that it was about 200-fold higher in the patient (52,263 ng/mL) than in healthy controls (263 ng/mL).

*Intracellular cytokines analysis.* To clarify the association between hypereosinophilia and hyperlymphocytosis, we analyzed intracellular cytokine expression in $CD3^-CD4^+$, $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ activated lymphocytes. As expected for cytotoxic lymphocytes, $CD3^+$ $CD4^-$ $CD8^+$ expressed IFNγ and TNFα, whereas $CD3^-$ $CD4^+$ expressed higher levels of IL-4, IL-13 than did $CD3^+$ $CD4^+$ cells, and surprisingly elevated levels of TNFα, IFNγ and IL-2. IL-5 expression was low but 4 fold higher in $CD3^-$ $CD4^+$ than in $CD3^+$ $CD4^+$ lymphocytes and no IL-6 was detected (Figure 2A). Overall, the cytokine profile of abnormal T cells was considered as Th0/Th2. Different patterns of T-cell phenotypes and cytokine profile have already been reported in patients with HES:




a

| Cluster of differentiation | Patient % positive lymphocytes | Normal values % positive lymphocytes |
|---|---|---|
| CD2 | **97.5** | 68-92 |
| CD5 | **97.7** | 58-85 |
| CD7 | 2.2 | 56-84 |
| CD3 | 1.9 | 60-87 |
| CD4 | **97.5** | 32-61 |
| CD8 | 0.5 | 14-43 |
| TCR αβ | 1.5 | 56-84 |
| TCR γδ | 0.1 | <5 |
| CD 16 | 0.6 | 4-28 |
| CD19 | 0.9 | 2-14 |
| CD27 | 2.8 | X |
| CD30 | 0.6 | <1 |
| CD45RO | 97 | 40-72 |
| CD45RA | 3.8 | 25-57 |
| CD62L | **93.4** | X |
| CD11b | 1.8 | X |
| CD25 | 1.4 | <8 |
| CD28 | **96.6** | X |
| HLA DR | **65** | 6-31 |
| CD95 | **96.7** | 10-62 |
| CD95L | 0.2 | X |
| Cytoplasmic CD3 | **90** | 60-85 |

b

X = Undetermined

**Figure 1: Triple-color CD3/CD4/CD8 and lymphocyte immunophenotypic analysis.**
**1 a. Detection of CD4+CD3- blood lymphocytes by triple-color flow cytometric analysis. Flow cytometric analysis of circulating lymphocytes was performed three times over an 8-month period without any significant modification of the various parameters. Data from a representative experiment obtained on more than 10,000 viable cells. Blood cells of the patient were stained simultaneously with FITC-conjugated anti-CD3, PE-conjugated anti-CD4 and PECY5-conjugated anti-CD8. The gating window was set to include the lymphoid population on size and structure criteria and two color flow cytometric analysis on the same window showed that these cells were CD14-CD45+. Numbers indicate the proportion of cells in each quadrant. Staining with matching negative controls (irrelevant murine Ig) conjugated with respective dyes yielded a homogeneous population of cells clustering in the left lower quadrant (not shown).**
**1 b: Lymphocyte immunophenotypic analysis. Phenotypic analysis of the blood lymphoid population of the patient was performed by flow cytometry and the percentages of positive lymphocytes for each marker are indicated. Normal values are indicated as reference. The gating window was set to include the lymphoid population on size and structure criteria and two-color flow cytometric analysis on the same window showed that these cells were CD14-CD45+.**


Figure 2A: Flow cytometric titration of intracytoplasmic cytokines. IL-2, IFN g, IL-5, IL-4, IL-6, IL-10, IL-13 and TNFa were measured by flow cytometry after permeabilization of CD3-CD4+, CD3+CD4+ and CD3+CD8+ circulating lymphoid cells.

Table 1.

| CYTOKINES (pg/mL) | Patient | |
|---|---|---|
| | RPMI Medium | PMA + Ionomycin |
| IL-5 | <1 | 702 |
| GM-CSF | 63.8 | 4671 |
| | Control subject | |
| | RPMI Medium | PMA + Ionomycin |
| IL-5 | <1 | 471 |
| GM-CSF | 12.7 | 810 |

$CD3^-$ $CD4^+$ and IL-$4^+$ IL-$5^+$ (7, 11) or IL-$5^+$ alone;[8] $CD3^+$ $CD4^-$ $CD8^-$ and IL-$5^+$ $GM^-$ $CSF^+$ (IL-$2^+$)[9] or IL-$5^+$ or IL-$5^+$ IL-$3^+$.[6] Sugimoto reported evolution for more than 13 years of hypereosinophilia associated with clonal $CD3^-$ $CD4^+$ lymphocytosis of Th2/Th0 type, with 44 % of the $CD4^+$ cells being IFN$\gamma^+$.[19] In the present patient, 90 % lymphocytes produced IFNg, in contrast to the previous study, in which IFNγ was barely detectable in $CD3^-$ $CD4^+$ cells in 3 of 4 cases.[11]

*Serum cytokine levels.* Serum cytokine levels from the patient were undetectable or near to the minimum quantifiable levels using the CBA kit (IL-2 <2.6 pg/mL, IL-4 <2.6 pg/mL, IL-5 <2.4 pg/mL, interferon γ7 pg/mL, TNFα <2.8 pg/mL, IL-10 <2.8 pg/mL). Although the abnormal lymphocytes secrete large amounts of Th0/Th2 cytokines, serum levels represent only the balance between production and consumption of cytokines.

*IL-5 and GM-CSF production by cultured PBMC. In vitro*, PMA (5 ng/mL) ionomycin (500 ng/mL) stimulated PBMC from the studied patient released larger amounts of IL-5 and GM-CSF into the supernatant within a 19-hour incubation period than did corresponding cells from a normal subject. (Table I). Spontaneously, unstimulated PBMC from the patient and control subject did not release IL-5; those from the patient released a low level of GM-CSF, whereas GM-CSF release from the healthy subjects unstimulated PMMC was not detectable. (Table I).

*T cell receptor rearrangement and clonality studies.* To determine the clonal pattern of this lymphoid population, we used a PCR amplification based on the rearrangement of the δ and γ TCR genes and IgH genes and amplified fluorescent products were analyzed. For our patient we observed only two peaks at x and y base-pairs generated using the VγIJ1J2 PCR, indicating the presence of either a monoclonal cell population with two different alleles or most probably T-cell biclonality. The absence of a normal Gaussian distribution was consistent with the very high purity of the clonal lymphocytes (Figure 2B).

*Karyotype*. Cytogenetic analysis was performed on cells from the lymph node and on PBMC isolated from venous blood. Unstimulated cells from the lymph node showed two metaphases in 24-hour culture : one 46, XY and the other 47, XY, +7. After 96 hours of culture with PHA, 20 metaphases were observed : 19 were 46, XY and one 47, XY, +7. PBMC stimulated by PHA or IL-2 produced only normal metaphases (46, XY). PBMC stimulated by PMA plus ionomycin produced 3 metaphases which were 47, XY , +7. PBMC were composed of 84% lymphocytes, 15.7% monocytes and 0.3% granulocytes. So, we suggest that trisomy of chromosome 7 was observed in the lymphocytes from the patient. Moreover, lymphoproliferative tests with mitogenic agents performed on PBMC, *in vitro*, did not induce proliferation with anti-CD3 and only low rates with PHA and PMA, whereas mitogenic combinations (PMA plus ionomycin, PMA plus IL-2 or PHA plus IL-2) induced an important level of lymphoproliferation (data not shown). Detection of a $CD3^-$ $CD4^+$ population and the presence of a chromosomal abnormality are consistent with the diagnosis of T-cell lymphoma. Trisomy 7 associated with hypereosinophilia has been described only once in a case of chronic eosinophilic leukemia.[21] Fluorescent *in situ* hybridization (FISH) using a chromosome 7 centromere probe on purified eosinophil and purified $CD3^-$ $CD4^+$ or $CD3^+$ $CD4^+$ lymphocytes might be useful to test the presence of the abnormality on different cell populations from the studied patient.

*Conclusion.* It has been proposed that, for some patients, the lymphocytic variant of HES could constitute the first step before development of a lymphoproliferative disorder.[11] The role the Th1/Th2 balance may play in progression from HES to lymphoma should be taken into account for prognosis and treatment. No

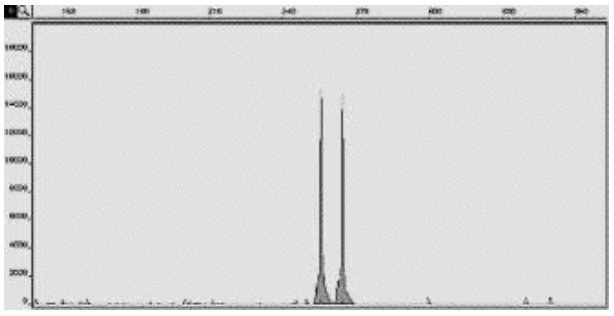


Figure 2B: T cell receptor rearrangement analysis. These data were generated using the VgIJ1J2 PCR for the gamma chain of the TCR and the fluorescent products were analyzed. The sample consisted of purified blood lymphocytes from the patient. Little peaks were size standards. There were two clonal TCR gamma specific peaks evident at 255 bp and 264 bp indicating the presence of a clonal T-cell population.

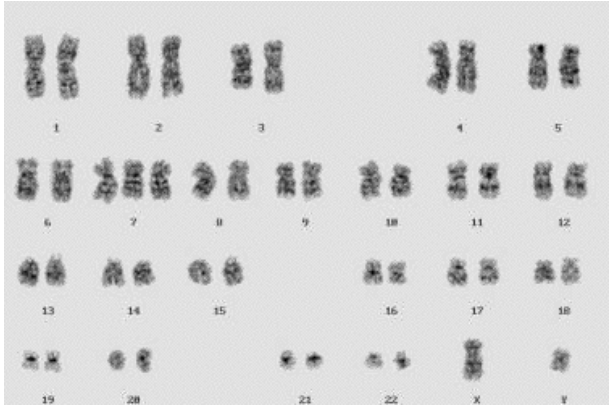


Figure 3. Karyotype analysis. Karyotype analysis showing the presence of isolated trisomy 7.

treatment was given to the studied patient because of his clinical stability. However, clinical and biological parameters (CD3/CD4/CD8 immunophenotyping, clonality analysis and measurement of serum TARC level) should be closely monitored. The 47,XY,+7 karyotype described here gives rise to the possibility that one or more genes located on chromosome 7 are involved in eosinophil homeostasis.

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*An ethics committee approved this study and the patient gave his informed written consent.*

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