

LETTER TO THE EDITOR

HIGH INTRACYTOPLASMATIC LEVELS OF IL-4 AND IL-5 IN A PATIENT WITH GLEICH'S SYNDROME: CASE REPORT

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Gleich's syndrome is characterized by recurrent localized angioedema, hypereosinophilia, elevated levels of IgM, rapid weight gain, itchy urticaria and fever. Little is known about the pathogenesis of this disease. Increased serum levels for IL5, IL6 and C5a have been reported before and during clinical exacerbations. In order to better understand the role of the T cells in Gleich's syndrome we analyzed the intracellular cytokine expression in CD3⁺ cells of a patient affected by the disease. As hypereosinophilia was documented, we asked whether IL-4 and IL-5 levels were increased, and the intracytoplasmatic expression of these Th2-cytokines was determined. The percentage of T lymphocytes (CD3-gated cells) of both CD8⁻ and CD8⁺ phenotype expressing different cytokines showed an unusually high percentage of Th2-related cytokine (IL-4, IL-5 and IL-13) expressing T lymphocytes. The two new variants (myeloproliferative and lymphoproliferative) seem to account for hypereosinophilia in patients with hypereosinophilic syndrome (HES). In the lymphoproliferative variant, the presence of a clonal CD3-CD4⁺ Th2 like lymphocyte secreting IL-4 and IL-5 in peripheral blood, may explain the hypereosinophilia and the hyper-IgE. In our study we show that the patient had a lymphoproliferative variant and her T cell had a Th2 type phenotype. Moreover, we suggest that Th2 lymphocytes may play a role in the pathogenesis of Gleich's syndrome. Further studies are needed to evaluate the possibility that a polyclonal aspecific activation of Th2 type cells can lead to hypereosinophilia, IgE production and the other manifestations typical of Gleich's syndrome.

Hypereosinophilic syndrome (HES) comprises a group of myeloproliferative disorders of unknown aetiology that are marked by sustained overproduction of eosinophils and tissue infiltration. Recently, a subgroup of patients (e.g. patients with episodic angioedema with eosinophilia, or with Gleich's syndrome) with profound eosinophilia characteristics of HES, but who do not go on to develop end organ damage, has been identified (1).

Gleich's syndrome was described in 1964 for the first time, and so far only about 20 patients have been recognized as having this syndrome (2). It is characterized by recurrent angioedema (face-neck-trunk-hands and feet), itchy urticaria and fever. Peripheral blood leukocytosis with marked eosinophilia (up to 6-7000 elements / mm³), elevated IgM and IgE levels, rapid weight gain (from 5% to 20% of the normal weight) are

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Table 1. Intracytofluorimetric analysis of cytokine production.

	CD3+CD8+ (normal values $\pm$ SD)	CD3+CD8- (normal values $\pm$ SD)
IL-2	26.4% (20.6 $\pm$ 16.1%)	58.8% (39.4 $\pm$ 18.6%)
IL-4	5.3% (1.8 $\pm$ 1.1%)	16.3% (2.4 $\pm$ 1.8%)
IL-5	6.6% (1.4 $\pm$ 0.7%)	17.1% (1.9 $\pm$ 0.8%)
IL-10	6.7% (1.2 $\pm$ 0.8%)	9.1% (2.1 $\pm$ 1.2%)
IL-13	8.0% (2.1 $\pm$ 1.0%)	19.0% (4.1 $\pm$ 0.4%)
TNF- α	5.7% (24.1 $\pm$ 19.3%)	21.1% (30.6 $\pm$ 18.3%)
IFN- γ	17.6% (25.5 $\pm$ 12.0%)	8.2% (15.8 $\pm$ 7.1%)

typically described during the attacks (3). Little is known about the pathogenesis of this disease, although certain hypotheses have been proposed. The symptoms are clearly related to the number of eosinophils in the peripheral blood. Biopsies of the affected skin suggest that activated eosinophils localize in the skin, release the granules and cause mast-cell degranulation with resultant angioedema (4). Increased serum levels for IL5, IL6 and C5a have been reported before and during clinical exacerbations of this syndrome (5). The increment serum IL-6 may be related to an increased production of blood monocytes and endothelial cells, which could be stimulated by eosinophil mediators during the acute phase of the disease. Moreover, an increased percentage of activated T cells 10 days before maximal eosinophilia has been reported, suggesting a role of the T lymphocytes in the pathogenesis of the disease (6). In order to better understand the role of the T cells in Gleich's syndrome, we analyzed the intracellular cytokine expression in CD3+ cells in a patient affected by the disease.

MATERIALS AND METHODS

A 19 year old female had recurrent episodes of angioedema for 4 years. At the age of 6 she suffered from herpetic dermatitis, and by the age of 12 she presented with chronic papulous dermatitis, primarily on her arms and legs. At the age of 16 she was hospitalized. Laboratory analyses revealed increased IgE levels (> 2000 KUI/ml), hypereosinophilia (eosinophils (EOS) 20%; and white

blood cells (WBC) 11000/ μ l). A cutaneous biopsy was performed in response to the recurrent angioedema of her face and neck. Lymphocytes, histiocytes and eosinophils were present in the skin specimen, which seemed to represent Dühring dermatitis. However, linear IgA deposits in the dermo-epidermic junction and anti-gliadin (AGA) and endomysial (EMA) antibodies were absent. Treatment with diphenylsulfone, cyclosporine and a diet without gluten were ineffective. Gleich's syndrome was then suspected and she was referred to our Department. We confirmed the presence of hypereosinophilia (18.9%; WBC 10930/ μ l) and observed the presence of leukocytosis (WBC 16820/ μ l), increased IgM levels: 251 mg/dl (40-230 mg/dl normal range n.r.); high amounts (> 15 μ g/L n.r.) of serum ECP: 184-360 μ g/L, with normal tryptase and finally, the presence of antibodies against smooth muscle (1:20; 1:80 type V). A decrease in C4 and C3c levels: 10-6 mg/dL (10-40 mg/dl n.r.) and 80-86 mg/dL (90-180 mg/dl n.r.) respectively, was also found, but quantitative and functional C1INH was normal (7).

Thyroidal, hypophyseal and suprarenal hormonal levels were within normal range. The morphology of peripheral blood and medullar eosinophils resulted normal. In order to distinguish between a myeloproliferative or a lymphoproliferative disorder, we looked for WT-1 specific mutation by RT-PCR of a bone marrow biopsy. A myelodysplastic syndrome was excluded because it proved negative (8). Moreover, the FIPLI/PDGFRa mutation's absence led us to exclude the possibility of clonal hematopoietic malignancies classified as chronic eosinophilic leukemias and mostly associated with HES (9). No heart (Electrocardiography and EcocolorDoppler were negative) nor gastrointestinal (abdominal ecography was negative), nor nervous (central and peripheral nervous system) involvement were found. She showed a progressive weight gain (up to 13 kilograms in 10 months). However, the body temperature was always within normal range. Moreover, the papulous dermatitis that she had on arms and legs, was not strictly in accordance with the syndrome. As hypereosinophilia was documented, we asked whether IL-4 and IL-5 levels were increased, and the intracytoplasmatic expression of these Th2-cytokines was determined in polyclonally stimulated blood lymphocytes of our patient.

Intracytofluorimetric analysis of IFN- γ , IL-4, IL-5, IL-2, IL-10 and IL-13 synthesis at single-cell level was performed as described (10). Briefly, 1×10^6 freshly isolated peripheral blood mononuclear cells (PBMC) were stimulated with phorbol myristate acetate (PMA) (10 ng/ml) plus ionomycin (1 μ M) for 4 h, the last 2 h in the presence of brefeldin A (5 μ g/ml). After incubation, the cells were washed twice with PBS pH 7.2, fixed for 15 min with 2% formaldehyde in PBS pH 7.2, washed twice

with 0.5% BSA in PBS pH 7.2, permeabilized with PBS pH 7.2 containing 0.5% BSA and 0.5% saponin, and then incubated with the specific mAb. The cells were analyzed on a FACSCalibur cytofluorimeter using CellQuest software (Becton Dickinson). The area of positivity was determined using an isotype-matched mAb (11).

RESULTS

The percentage of T lymphocytes (CD3-gated cells) of both CD8⁺ and CD8⁺ phenotype expressing different cytokines are reported in Table I. A higher percentage of CD3⁺ cells expressing Th2-related cytokine (IL-5, IL-4, IL-13, IL-10) and a relatively lower percentage of Th1-related cytokine interferon-gamma (INF- γ), tumor necrosis factor (TNF α) were found (Table I).

DISCUSSION

HES is defined as a persistent eosinophilia of unknown aetiology complicated by organ damage or dysfunction. Although this entity was introduced in an attempt to standardize management of patients, the heterogeneous nature of HES rapidly emerged with regard to clinical and biological parameters, disease course and response to therapy. Recent observations indicate that at least two distinct haematological disorders, involving the myeloid and the lymphoid lineages respectively, account for hypereosinophilia in patients who meet the diagnostic criteria of HES: the myeloproliferative variant and the lymphoproliferative variant (7-12). Patients with clinical and biological markers reminiscent of myeloproliferative disorders generally respond poorly to steroids and present severe cardiac complications.

In contrast, in the lymphoproliferative variant, high IgE levels and polyclonal hypergammaglobulinemia are considered to be markers of good prognosis as they are frequently associated with the restriction of clinical manifestations to the skin and a favourable response to glucocorticoid therapy (11). In patients with the lymphoproliferative variant of HES, the presence has been described of clonal CD3-CD4⁺ Th2 like lymphocytes secreting IL-4 and IL-5 in peripheral blood, that may explain the hypereosinophilia and the hyper-IgE (7-13). In those CD3-CD4⁺ Th2 like lymphocyte chromosomal abnormalities have been demonstrated that predispose

to lymphoma development (7-12). CD3-CD4⁺ Th2 like lymphocytes have been demonstrated in 2 cases of Gleich syndrome, but the role of lymphocytes in most cases of Gleich's syndrome, where the CD3-CD4⁺ Th2 like lymphocyte clones have not been demonstrated, has not yet been clarified. In our study we show that the T cell of our patient had a Th2 type phenotype. It is known that Th2-related cytokines are important for IgE overproduction and hypereosinophilia. Our study suggests that Th2 lymphocytes may play a role in the pathogenesis of Gleich's syndrome, and further studies are needed to evaluate the possibility that a polyclonal and specific activation of Th2 type cells can lead to hypereosinophilia, IgE production and the other manifestations typical of Gleich's syndrome.

REFERENCES

1. **Ackerman S.J. and J.H. Butterfield.** 2000. Eosinophilia, eosinophil-associated diseases and hypereosinophilic syndrome. *Hoffman R. "Haematology: basic principles and practice"*, 3rd Ed. Chapter 40.
2. **Gleich G.J., A.L. Schroeter, P. Marcoux, M.I. Sachs, E.J. O'Connell and P.F. Koheler.** 1984. Episodic angioedema associated with eosinophilia. *N. Engl. J. Med.* 310:1621.
3. **Tomoe S. and I. Iwamoto.** 1993. Episodic angioedema with eosinophilia. *Nippon Rinsho* 51: 795.
4. **Gleich G.J. and D.A. Loegering.** 1984. Immunobiology of eosinophils. *Ann. Rev. Immunol.* 2:429.
5. **Nakashima K., T. Sakurada, S. Imayama, S. Masukawa, J.A. Ember, T.E. Hugli and M. Abe.** 1998. A case of episodic angioedema associated with blood eosinophilia; upregulated C5a receptor expression on eosinophils. *Allergy* 53:320.
6. **Butterfield J.H., K.M. Leiferman, J. Abrams, J.E. Silver, J. Bower, N. Gonchoroff and G.J. Gleich.** 1992. Elevated serum levels of interleukin-5 in patients with the syndrome of episodic angioedema and eosinophilia. *Blood* 79:688.
7. **Roufosse F., L. Schandene, C. Sibille, K. Willard-Gallo, B. Kennes, A. Eflra, M. Goldman and E. Cogan.** 2000. Clonal Th2 lymphocytes in patients with the idiopathic hypereosinophilic syndrome. *Br:*

J. Haematol. 109:540.

8. **Cilloni D., E. Gottardi, F. Messa, M. Fava, P. Scaravaglio, M. Bertini, M. Girotto, C. Marinone, D. Ferrero, A. Gallamini, A. Levis and G. Saglio; Piedmont Study Group on Myelodysplastic Syndromes.** 2003. Significant correlation between the degree of WT1 expression and the International Prognostic Scoring System Score in patients with myelodysplastic syndromes. *J. Clin. Oncol.* 21:1988.
9. **Cools J., E.H. Stover, I. Wlodarska, P. Marynen and D.G. Gilliland** 2004. The FIP1L1-PDGFRalpha kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia. *Curr. Opin. Hematol.* 11:51.
10. **Maggi E., M.G. Giudizi, R. Biagiotti, F. Annunziato, R. Manetti, M.P. Piccininni, P. Parrochi, S. Zampognaro, L. Giannarini, G. Zuccati and S. Romagnani.** 1994. Th2-like CD8+ T cells showing B cell helper function and reduced cytolytic activity in human immunodeficiency virus type 1 infection. *J. Exp. Med.* 180:489.
11. **Cosmi L., F. Annunziato, M. Iwaasaki, G. Galli, R. Manetti, E. Maggi, K. Nagata and S. Romagnani.** 2000. CRTH2 is the most reliable marker for detection of circulating human type 2 Th and type 2 T cytotoxic cells in health and disease. *Eur. J. Immunol.* 30:2972.
12. **Morgan S.J., H.M. Prince, D.A. Westerman, C. McCormack and I. Glaspole.** 2003. Clonal T-helper lymphocytes and elevated IL-5 levels in episodic angioedema and eosinophilia (Gleich's syndrome). *Leuk. Lymphoma* 44:1623.
13. **Cogan E., L. Schandene, A. Crusiaux, P. Cochaux, T. Velu and M. Goldman.** 1994. Brief report: clonal proliferation of type 2 helper T cells in a man with the hypereosinophilic syndrome. *N. Engl. J. Med.* 24:330.