

Sergei A. Kharitonov, MD, PhD^b
Peter J. Barnes, DM, DSc, FRS^b
Vibeke Backer, MD, DMSci^a

From ^athe Respiratory and Allergy Research Unit, Department of Respiratory Medicine L, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark and ^bthe Airway Disease Section, National Heart and Lung Institute, Imperial College London, United Kingdom. E-mail: lars.pedersen@dadlnet.dk.

Supported by research grants from Team Denmark; Anti-Doping Denmark; the Health Insurance Foundation; and the Academy of Muscle Biology, Exercise and Health Research. Polar Electro Denmark ApS (Denmark) provided the Polar FS3 devices and Flaemnuova (Italy) provided the Easyneb II devices.

Disclosure of potential conflict of interest: L. Pedersen has received financial support from the Academy of Muscle Biology, Exercise and Health Research, Anti-Doping Denmark, Team Denmark, the Health Insurance Foundation (Sygekassernes Helsefond), and Merck Sharpe & Dome. T. K. Lund has received financial support from Anti-Doping Denmark, Team Denmark, the Research Foundation of Bispebjerg Hospital, the Beckett Foundation, and Pharmaxis Ltd. P. J. Barnes has received research grants from GlaxoSmithKline, AstraZeneca, and Novartis. V. Backer has received financial support from Pharmaxis Ltd and the Danish Lung Foundation. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

- Pedersen L, Lund TK, Barnes PJ, Kharitonov SA, Backer V. Airway responsiveness and inflammation in adolescent elite swimmers. *J Allergy Clin Immunol* 2008;122:322-7.
- Bonsignore MR, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, et al. Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L668-76.
- Bonsignore MR, Morici G, Riccobono L, Profita M, Bonanno A, Paterno A, et al. Airway cells after swimming outdoors or in the sea in nonasthmatic athletes. *Med Sci Sports Exerc* 2003;35:1146-52.
- Morici G, Bonsignore MR, Zangla D, Riccobono L, Profita M, Bonanno A, et al. Airway cell composition at rest and after an all-out test in competitive rowers. *Med Sci Sports Exerc* 2004;36:1723-9.
- Helenius IJ, Ryttila P, Metso T, Haahela T, Venge P, Tikkanen HO. Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers. *Allergy* 1998;53:346-52.
- Belda J, Ricart S, Casan P, Giner J, Bellido-Casado J, Torrejon M, et al. Airway inflammation in the elite athlete and type of sport. *Br J Sports Med* 2008;42:244-9.
- Boulet LP, Turcotte H, Langdeau JB, Bernier MC. Lower airway inflammatory responses to high-intensity training in athletes. *Clin Invest Med* 2005;28:15-22.
- Fitch KD, Sue-Chu M, Anderson SD, Boulet LP, Hancox RJ, McKenzie DC, et al. Asthma and the elite athlete: summary of the International Olympic Committee's consensus conference, Lausanne, Switzerland, January 22-24, 2008. *J Allergy Clin Immunol* 2008;122:254-60.
- Anderson SD, Kippelen P. Airway injury as a mechanism for exercise-induced bronchoconstriction in elite athletes. *J Allergy Clin Immunol* 2008;122:225-37.
- Bernard A, Carbonnelle S, de Burbure C, Michel O, Nickmilder M. Chlorinated pool attendance, atopy, and the risk of asthma during childhood. *Environ Health Perspect* 2006;114:1567-73.
- Carraro S, Pasquale MF, Da Fre M, Rusconi F, Bonetto G, Zanconato S, et al. Swimming pool attendance and exhaled nitric oxide in children. *J Allergy Clin Immunol* 2006;118:958-60.

doi:10.1016/j.jaci.2008.11.039

Successful treatment of chronic drug-resistant urticaria with alprazolam

To the Editor:

Alprazolam has shown competitive antagonism on muscarinic and histamine H1 receptors and noncompetitive antagonism on histamine H2 receptors. The antagonistic effect of alprazolam on histamine H1 receptors has been studied in guinea pig ileum, and that on histamine H2 receptors has been studied on rat uterus.¹ Pretreatment with kadsurenone or alprazolam improved survival in hypersensitivity reactions to ovalbumin in guinea pig lung parenchymal strips and in guinea pigs *in vivo*.² This antihistaminic effect has not been tested in allergic patients, although one study found that alprazolam appeared to be safe and effective for the

treatment of anxiety and depression in adolescents with asthma.³ Exceptionally, alprazolam might induce allergic symptoms and angioedema.⁴

Chronic urticaria (CU) is a common disorder that is generally of unknown origin, although the cause might be autoimmune in some cases.⁵ In some patients severe urticaria is a distressing and disabling condition⁶ with a significant effect on the quality of life, and patients often receive various unsuccessful treatments. First-line treatment consists of the newer-generation oral antihistamines, whereas other drugs might be considered in refractory cases,⁷ although more controlled studies are necessary.

We recently studied allergic sensitization in drug abusers, who are often treated with alprazolam. Forty-two drug abusers were tested with common allergens (aeroallergens and foods) and pharmacologic drugs (nonsteroidal anti-inflammatory drugs and other suspected drugs). Histamine (10 mg/mL) was used as a positive control, and a wheal area greater than 7 mm² (an area measuring 3 × 3 mm) was considered positive. In some patients skin prick test responses with allergens were negative, but results with histamine, used as a positive control, were also totally negative. This is unusual because the histamine control should elicit a small wheal, even when the patient has taken an antihistamine. These patients denied intake of antihistamines or other antiallergic drugs, but all were taking 2 mg of alprazolam (Trankimazin; Pfizer S. A., Madrid, Spain) 3 times daily. We then decided to carry out a preliminary study to investigate whether alprazolam could be useful in severe refractory CU.

We identified 558 patients with idiopathic CU in our database of 19,736 patients followed in the allergy section during the last 20 years. All patients had undergone a protocol investigation according to recent studies^{5,6} to investigate the possible cause. Only 12 of the 558 patients had criteria of severe refractory idiopathic urticaria of more than 10 years of evolution in which treatment with all known drugs used in this disease (all first-generation antihistaminics,⁸ all newer-generation antihistamines, corticosteroids in different schemes, cyclosporine treatment,⁹ intravenous gammaglobulin,¹⁰ and omalizumab¹¹) had failed. The 12 patients were initially randomized to 2 mg of alprazolam 3 times daily or 10 mg of rupatadine (Rupafin; Uriach, Barcelona, Spain) 3 times daily, a new antihistamine platelet-activating factor (PAF) inhibitor. The drugs were introduced into identical empty capsules and administered randomly by a nurse blinded to the contents. However, only 8 patients finally consented to participate and completed the trial.

After 2 days, the urticaria disappeared in the 6 patients treated with alprazolam and persisted in the 2 patients treated with rupatadine. No secondary effects were observed. Urticaria reappeared after 36 hours after withdrawal of alprazolam and improved 8 hours after readministration.

Although both alprazolam and rupatadine are PAF receptor antagonists,^{1,2} the poor response to rupatadine suggests that the main mechanism of alprazolam in CU might be blockade of histamine H1 receptors and not PAF inhibition. The importance of this small study lies in the fact that 6 of 6 patients treated with alprazolam responded, whereas the 2 patients treated with rupatadine did not.

Because of the difficulty in identifying the causative factors involved, treatment for severe CU often focuses on measures to provide symptomatic relief. Our positive experience, although only in a small group of patients, suggests a controlled trial with

alprazolam in a large group of patients with drug-resistant CU is necessary.

Antonio Dueñas-Laita, MD, PhD^a
Pedro Ruiz-Muñoz, MD^b
Alicia Armentia, MD, PhD^c
Florentino Pinacho, DUE^d
Blanca Martín-Armentia, PhD^e

From ^aServicio de Farmacología Clínica–Unidad Regional de Toxicología Clínica, Hospital Universitario Río Hortega; ^bAsociación Castellano Leonesa de Ayuda a Drogodependientes; and ^cSección de Alergia, Hospital Universitario Río Hortega, Valladolid, Spain. E-mail: aliciaarmentia@gmail.com.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

REFERENCES

1. Alvarez FJ, Velasco A, Palomares JL. Blockade of muscarinic, histamine H1 and Histamine H2 receptors by antidepressants. *Pharmacology* 1988;37:225-31.
2. Darius H, Smith JB, Lefer AM. Inhibition of the platelet activating factor mediated component of guinea pig anaphylaxis by receptor antagonists. *Int Arch Allergy Appl Immunol* 1986;80:369-75.
3. De Vance CL, Hill M, Antal EJ. Therapeutic drug monitoring of alprazolam in adolescents with asthma. *Ther Drug Monit* 1998;20:257-60.
4. Sellas-Dupre G, Nieto Lopez M, Garcia Vicente JA, Salvador-Chiva J. Alprazolam-induced tongue angioedema. *Pharmacopsychiatry* 2006;39:154-6.
5. Hide M, Francis DM, Gratton CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-604.
6. Deacock SJ. An approach to the patient with urticaria. *Clin Exp Immunol* 2008;153:151-61.
7. Morel V, Hauser C. Chronic urticaria. *Rev Med Suisse* 2008;4:1019-23.
8. Kaplan AP. Chronic urticaria: pathogenesis and treatment. *J Allergy Clin Immunol* 2004;114:465-74.
9. Serhat Inaloz H, Ozturk S, Akcali C, Kirtak N, Tarakcioglu M. Low-dose and short-term cyclosporine treatment in patients with chronic idiopathic urticaria: a clinical and immunological evaluation. *Dermatology* 2008;35:276-82.
10. Pereira C, Tavares B, Carrapatoso I, Loureiro G, Faria E, Machado D, et al. Low-dose intravenous gammaglobulin in the treatment of severe autoimmune urticaria. *Eur Ann Allergy Clin Immunol* 2007;39:237-42.
11. Spector SL, Tan RA. Omalizumab also successful in chronic urticaria. *J Allergy Clin Immunol* 2008;121:784-5.

doi:10.1016/j.jaci.2008.12.005

Signal transducer and activator of transcription 5 tyrosine phosphorylation for the diagnosis and monitoring of patients with severe combined immunodeficiency

To the Editor:

T⁻B⁺ natural killer (NK)⁻ severe combined immunodeficiency (SCID) is frequently caused by defects in the common γ chain (γ c) or the tyrosine kinase Janus kinase (JAK) 3. However, not all γ c or JAK3 SCIDs present with this typical phenotype because some might have low numbers of T cells, NK cells, or both. Other forms of SCID, such as IL-7 receptor alpha (IL-7R α) SCID, might present in a similar manner.¹ A rapid screen to distinguish among these patients is essential to direct genetic analysis and initiate appropriate therapy.²

Signal transducer and activator of transcription (STAT) 5 is a member of the STAT family of transcription factors. STATs are downstream of a number of cytokine and growth factor receptors, including γ c receptors. In healthy individuals IL-2 binds a trimeric receptor consisting of the IL-2 α , β , and γ chains. This receptor complex then trimerizes, bringing together the tyrosine kinases JAK1 and JAK3. These cross-phosphorylate each other, resulting in their activation and enabling them to phosphorylate STAT5. Once phosphorylated, STAT5 dimerizes, translocates to

the nucleus where it binds a defined DNA sequence, and activates transcription.³

Detection of tyrosine-phosphorylated STAT5 after IL-2 stimulation is therefore an indication of a functional IL-2/JAK3 signal transduction pathway, and abnormalities in STAT5 tyrosine phosphorylation might identify patients with defects in this pathway. Historically, STAT5 activation has been assessed by using the electrophoretic mobility shift assay (EMSA). EMSA specifically detects STAT5 binding to its consensus DNA sequence³; however, EMSA is time-consuming and requires large volumes of blood.

A functional STAT5 tyrosine phosphorylation (ptyr) assay was established to detect abnormalities in signaling through the γ c/JAK3 pathway. Ethical approval and consent from parents/guardians was obtained for patients included in the study. Immunophenotyping by means of flow cytometry was performed with standard techniques to determine T, B, and NK cell numbers and γ c expression,² and phosphorylated STAT5 was detected by means of EMSA⁴ and flow cytometry (FACS). To detect STAT5 ptyr by means of FACS, 100 μ L of blood was left unstimulated or stimulated with 10⁴ units of IL-2, lysed, fixed, washed, and permeabilized before 5 μ L of antibodies (STAT5 ptyr, CD19 phycoerythrin, and CD4 peridinin-chlorophyll-protein complex; BD Biosciences, San Jose, Calif) was added. Ten thousand lymphocyte events were acquired and analyzed.

STAT5 ptyr by means of FACS was compared with results obtained from staining for γ c by means of flow cytometry, expression of JAK3 by means of immunoblotting, available genetic results, and detection of STAT5 by using EMSA. Fig 1 is a representative experiment and shows γ c expression by FACS (Fig 1, A) in γ c-deficient SCID (patient 8 in Table I) in comparison with that seen in a healthy control subject and demonstrates the correlation between a STAT5 EMSA (Fig 1, B) and STAT5 ptyr (Fig 1, C) by means of flow cytometry. By using STAT5 ptyr analysis, there is a significant shift in fluorescence in the normal blood control compared with the patient sample (Fig 1, C). By means of EMSA analysis (Fig 1, B), a large band could be detected in the control subjects where phosphorylated STAT5 had bound to the oligonucleotide; the specificity of this band was demonstrated by adding unlabeled oligonucleotide, which competed most of the protein away from the labeled oligonucleotide. This band was faint or undetectable when unlabeled oligonucleotide was added in the patient sample. The faint remaining band is likely to be due to activation of STAT5 by γ c-independent signaling pathways, such as prolactin.

Table I summarizes the data from 32 patients with SCID. Twelve γ c and 4 JAK3 genetically defined SCIDs, including several patients who had atypical immunophenotypes (T^{low}B⁺NK⁺), had absent or highly abnormal STAT5 tyrosine phosphorylation in their lymphocytes. In 2 patients who had high levels of maternal engraftment (patients 12 and 14), a negative STAT5 ptyr peak was present, as well as a smaller positive STAT5 ptyr peak presumed to be due to maternal cells (Fig 1, C). A further patient with a missense mutation allowing partial cytokine binding (patient 5) showed abnormal but not absent STAT5 ptyr. In patient 17 there was absent IL-2–induced STAT5 ptyr, but a confirmed γ c or JAK3 mutation has not been identified, although a number of sequence variants have been identified in his JAK3 gene. A second affected child carried these same variants, whereas an unaffected sibling did not. Forty control samples and 15 samples from other forms of SCID or undefined combined