



FIG 1. Patients with APECED have fewer T lymphocytes bearing regulatory phenotype. Peripheral blood CD4⁺ cells from *AIRE*^{-/-} patients and age-matched controls were assessed by flow cytometry for cell surface markers CD4/CD25 (A) or CD4/CD25/GITR (B). Lines represent medians; in (A), the right Y axis applies to the right panel. *FOXP3* transcripts for total CD4⁺ cells were quantified relative to *GAPDH* by RT-PCR (C). Pts, Patients; ctrl, controls.

of Treg cells is also suggested by data showing that self-antigen displayed on thymic epithelial cells induces increased production of Treg cells.¹⁰ A further indication that AIRE may play a role in the generation of Treg cells comes from certain striking similarities observed in organ-specific autoimmune manifestations developed in humans and mice with APECED and immune deficiency, polyendocrinopathy X-linked syndromes (IPEX), lacking the *AIRE/aire* or *FOXP3/foxp3* gene, respectively.⁶

Taken together, our data demonstrate that in patients with APECED lacking a functional *AIRE* gene, cells expressing markers characteristic of CD4⁺CD25⁺ Treg cells in peripheral blood are present but markedly decreased. *FOXP3* expression was not significantly altered, consistent with Treg cells being present, albeit at significantly lower levels. The role of these cells in the autoimmune phenomenon seen in patients with APECED remains to be investigated. These findings, although not conclusive, suggest that the *AIRE* gene may have a role in the generation of naturally occurring CD4⁺CD25⁺ Treg cells.

Kelli R. Ryan, PhD^a

Catherine A. Lawson, MRCP^b

Alice R. Lorenzi, MRCP^a

Peter D. Arkwright, PhD^c

John D. Isaacs, PhD^a

Desa Lilic, PhD^a

^aDepartment of Rheumatology

Faculty of Medical Sciences

Newcastle University

Newcastle upon Tyne NE2 4HH

United Kingdom

^bMolecular Medicine Unit

St James's University Hospital

Leeds, United Kingdom

^cAcademic Unit of Child Health

Booth Hall Children's Hospital

University of Manchester

Manchester, United Kingdom

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REFERENCES

- Lilic D. New perspectives on the immunology of chronic mucocutaneous candidiasis. *Curr Opin Infect Dis* 2002;15:143-7.
- Anderson MS, Venanzi E, Klein L, Chen Z, Berxins SP, Shannon T, et al. Projection of an immunological self shadow within the thymus by Aire protein. *Science* 2002;298:1395-401.
- Liston A, Lesage S, Wilson J, Peltonen L, Goodnow CC. Aire regulates negative selection of organ-specific T cells. *Nat Immunol* 2003;4:350-4.
- Su MA, Anderson MS. Aire: an update. *Curr Opin Immunol* 2004;16:746-52.
- Villasenor J, Benoist C, Mathis D. Aire and APECED: molecular insights into an autoimmune disease. *Immunol Rev* 2005;204:156-64.
- Fotnot JD, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat Immunol* 2005;6:331-7.
- Ramsey C, Winqvist O, Puhakka L, Halonen M, Moro A, Kampe O, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum Mol Genet* 2002;11:397-409.
- Montagnoli C, Bacci A, Bozza S, Gaziano R, Mosci P, Sharpe AH, et al. B7/CD28 dependent CD4+CD25+ regulatory T cells are essential components of the memory-protective immunity to *Candida albicans*. *J Immunol* 2003;169:6298-308.
- Lilic D, Gravenor I, Robson N, Lammas DA, Drysdale P, Calvert JE, et al. Deregulated production of protective cytokines to *Candida albicans* in patients with chronic mucocutaneous candidiasis. *Infect Immun* 2003;71:5690-9.
- Apostolou I, Sarukhan A, Klein L, von Boehmer H. Origin of regulatory T cells with known specificity for antigen. *Nat Immunol* 2002;3:756-61.

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False-elevated serum tryptase assay result caused by heterophilic antibodies

To the Editor:

The interference by human antianimal antibodies in immunoassays is a problem of growing concern.¹ Such antibodies, when reactive with animal IgG, can have a variable effect on immunoassays that use antibodies, raised in these animals or produced *in vitro* as mouse mAbs. In sandwich assays, the analyte forms a bridge between the capture and the detecting antibody. The concentration of analyte in a sample is proportional to the amount of bound detecting antibody, which is often labeled by an enzyme. Antianimal IgG antibodies in a sample can bridge the capture and detection antibodies in the absence of the analyte, resulting in a false-positive assay result. Most reports of antibody interference in immunoassays have been false increases, but negative interference by steric hindrance of antigen binding is also possible.²

TABLE I. Effect of HBT-treatment on serum tryptase

Patient	Tryptase ($\mu\text{g/L}$)	Tryptase + HBT ($\mu\text{g/L}$)	Sample date
1	110	ND	October 27, 2003
1	90.3	ND	October 31, 2003
1	49.5	ND	January 26, 2004
1	30.6	ND	April 20, 2004
1	42.3	2.9	August 13, 2004
1	61.6	4.5	December 6, 2004
1	52.2	4.6	January 31, 2005
2	42.8	40.2	
3	59.5	57.2	
4	66.4	61.1	
5	82.6	80.5	
6	100	91.4	
7	221	>200	
8	4.8	3.6	
9	7.0	11.1	
10	4.4	5.4	
11	1.7	2.4	

+ HBT, Serum preincubated in HBT; ND, not done, serum no longer available.

The administration of mouse mAbs for therapeutic and imaging purposes will lead in many patients to the formation of human antimouse antibodies (HAMAs). Such antibodies have a strong affinity for mouse IgG and are a major cause of immunoassay interference.³

Kaplan and Levinson³ distinguish specific HAMAs from heterophilic antibodies: human antibodies of low avidity that show broad activity against immunoglobulins from several species.

Human antianimal antibodies can also arise as a result of occupational exposure of farm workers, the presence of domestic animals in the home environment,² or ingestion of foods like cow's milk.⁴

Measurement of serum tryptase is an important test to perform in patients with suspected systemic mastocytosis.⁵ We report the presence of a false-elevated serum tryptase level that normalized after preincubation of the serum with a blocking reagent (Heterophilic Blocking Tubes [HBTs], Scantibodies Laboratory, Inc, Santee, Calif).

Tryptase in serum was determined with the UniCAP tryptase fluor-enzyme-immunoassay on the UniCAP 100 instrument according to the manufacturer's instructions (Pharmacia, Woerden, The Netherlands). In this sandwich assay, intact mouse mAbs are used as both capture and detecting antibodies. The directions for use of this tryptase assay report 11.4 $\mu\text{g/L}$ as 95% upper percentile value for healthy children and adults. HBTs contain predispensed and lyophilized specific binders to inactivate antianimal antibodies. For analysis of immunoassay interference, sera were incubated for 1 hour in HBTs at room temperature according to the manufacturer's instructions (www.scantibodies.com).

Patient 1 is a 23-year-old man who presented with skin lesions and severe gingivitis. During his study, he had worked on a farm. On examination, small red lesions were seen on his lower legs. Laboratory analysis showed a markedly increased erythrocyte sedimentation rate and elevated cholestatic and parenchymatous liver enzymes.

Because the treating physician believed the skin lesions to be urticaria pigmentosa, a serum tryptase measurement was requested and found to be markedly and repeatedly increased (Table I). However, there were no signs of mastocytosis in a biopsy taken (November 2003) from one of the lesions and in a concurrent bone marrow examination. The skin biopsy showed a perivascular infiltrate of lymphocytes and granulocytes and a normal amount of diffuse tryptase-staining mast cells. Subepidermal clusters of amorphous structures were seen that stained strongly for IgA and IgM. No mast cells were seen in a bone marrow smear. A hematological malignancy was excluded. The patient has never had an anaphylactic reaction. The elevated tryptase levels remained unexplained at that time. His final diagnosis (2005) was chronic autoimmune hepatitis accompanied by leukocytoclastic vasculitis.

The unexplained persistently elevated serum tryptase levels in patient 1 suggested us to consider immunoassay interference as a cause of false-positive assay results. Indeed, preincubation of 3 serum samples from patient 1 in HBTs reduced the tryptase result more than 10-fold, well into the reference range (Table I). Preincubation in HBTs of 6 other sera with elevated serum tryptase levels and 4 sera with normal tryptase levels, selected from samples submitted for routine tryptase analysis, did not substantially change the apparent tryptase levels (Table I).

In conclusion, this report shows that the serum tryptase assay is sensitive to immunoassay interference by heterophilic antibodies. In cases of unexplained elevated serum tryptase levels, immunoassay interference should be considered. Such interference can be effectively analyzed with HBT or other blocking reagents.^{2,4}

Albert W. van Toorenbergen, PhD^a

Paul L. A. van Daele, MD, PhD^b

Joke G. Boonstra, PhD^a

^aDepartment of Clinical Chemistry
Erasmus University Medical Center
Office L-139

Molewaterplein 40
3015 GD Rotterdam
The Netherlands

^bDepartment of Internal Medicine
Erasmus University Medical Center
Rotterdam, The Netherlands

REFERENCES

1. Bjerner J, Børner OP, Nustad K. The war on heterophilic antibody interference. *Clin Chem* 2005;51:9-11.
2. Kricka LJ. Human anti-animal antibody interferences in immunological assays. *Clin Chem* 1999;45:942-56.
3. Kaplan IV, Levinson SS. When is a heterophile antibody not a heterophile antibody? *Clin Chem* 1999;45:616-8.
4. Andersen DC, Koch C, Jensen CH, Skjødt K, Brandt J, Teisner B. High prevalence of human anti-bovine IgG antibodies as the major cause of false-positive reactions in two-site immunoassays based on monoclonal antibodies. *J Immunoassay Immunochem* 2004;25:17-30.
5. Valent P, Sperr WR, Schwartz LB, Horny HP. Diagnosis and classification of mast cell proliferative disorders: delineation from immunologic diseases and non-mast cell hematopoietic neoplasms. *J Allergy Clin Immunol* 2004;114:3-11.

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