Clinical Immunology (2006) **120**, 156–162

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Intact T cell responses in ataxia telangiectasia

Farzana Pashankar [a], Vibha Singhal [b], Ike Akabogu [b], Richard A. Gatti [c], Frederick D. Goldman [a,*]

[a] *Department of Pediatrics, Yale University School of Medicine, New Haven, CT 06520, USA*
[b] *University of Iowa College of Medicine, Iowa City, IA 52242, USA*
[c] *University of California Los Angeles, CA 90095, USA*

Received 17 January 2006; accepted with revision 13 April 2006
Available online 8 June 2006

KEYWORDS
Ataxia telangeictasia;
T cell antigen receptor;
T cell cytokines;
ATM mutations

Abstract Ataxia telangiectasia (A-T) is an autosomal recessive multisystem disorder associated with a variable immune deficiency. The mechanism for this remains unclear. Qualitative and quantitative defects of cellular immunity have been previously reported. However, despite laboratory evidence of significant immune abnormalities, opportunistic infections are uncommon. To address this discrepancy, we analyzed cytokine production by quantitative real-time PCR and T cell function at the single cell level by flow cytometry in four A-T patients. CD4 and CD8 T cell subsets from these patients displayed intact signaling in response to anti-CD3 stimulation, similar to controls. Stimulated T cells from A-T patients also produced normal to increased levels of Th1 (IL-2, IFN-γ) and Th2 (IL-10, IL-4) cytokines, relative to control values. Our results suggest that T cells from A-T patients may be more functionally intact than previously observed. This helps to explain the paucity of opportunistic infections encountered, unlike that encountered in other primary immunodeficiencies.

Introduction

Ataxia telangiectasia (A-T) is an autosomal recessive multisystem disorder characterized by progressive cerebellar ataxia, oculocutaneous telangiectasias, recurrent sinopulmonary infections, and a high incidence of lymphoid malignancies [1,2]. In 1995, the ATM gene was identified as the gene responsible for clinical disease [3,4]. The protein product of the ATM gene is a serine–threonine kinase involved in cell cycle control, gene regulation, intracellular signaling, telomere maintenance, and repair of double stranded DNA breaks [3,5].

Immune deficiency in A-T patients is quite variable, generally involving both humoral and cellular immune responses [2,6]. Humoral immune defects previously reported include reduction of serum IgA, IgE, IgG, as well as IgG subclass and functional antibody deficiencies [7–10]. Cellular immune dysfunction may be a function of the relative T cell lymphopenia, although qualitative defects have also been observed, including decreased proliferative responses to recall antigens and mitogens [9–15]. A-T patients also have an increased incidence of sinopulmonary infections, particularly with advancing age. This may in part be due to their poor antibody response to pneumococcal antigens [16]. However, despite laboratory evidence of significant immune abnormalities, both quantitative and qualitative, systemic bacterial and opportunistic infections and severe viral infections are surprisingly uncommon. In a recent retrospective study of 100 A-T patients, no complica-

* Corresponding author. Department of Pediatrics, Division of Hematology/Oncology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242, USA. Fax: +1 319 356 7659.
E-mail address: frederick-goldman@uiowa.edu (F.D. Goldman).

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doi:10.1016/j.clim.2006.04.568

tions were noted following live viral vaccination [10]. Additionally, pneumocystis carinii pneumonia, the hallmark infection of CD4 lymphopenia and T cell dysfunction, was not observed. To explain the relative discrepancy of laboratory immune abnormalities with the lack of significant clinical symptomatology, we analyzed cytokine production by quantitative real-time PCR (Q-PCR) and T cell function, at the single cell level, by flow cytometry in four patients with A-T. Although all patients were T lymphopenic, T cells were able to signal properly through their CD3 receptor, and Th1/Th2 cytokine levels were in the normal to slightly elevated range. Our data suggest that the integrity of T cell function in A-T may be more intact than previously recognized.

Materials and methods

Subjects

Four patients with A-T were identified from the Pediatric Immune Disorders Clinic at the University of Iowa. Diagnosis was suggested by characteristic neurological features (ataxia, oculomotor abnormalities, and dysarthria) and confirmed by laboratory abnormalities, including increased alpha-fetoprotein, increased radiation-induced chromosomal breakage, increased radiosensitivity of cells, as measured by colony survival [17], and absence of ATM protein by western blotting [18]. Informed consent was obtained from patients and parents prior to study entry. Charts were reviewed and families were interviewed for a detailed history, with emphasis on infections and immunization history.

Lymphocyte separation and immunophenotyping

For the immune analyses in this study, blood was collected from patients and healthy controls in heparinized tubes. Peripheral blood lymphocytes (PBL) were isolated by Ficoll-Hypaque gradient centrifugation. Following isolation, cells were plated at a density of $1-2 \times 10^6$/ml and incubated in tissue culture flasks at 37°C in 5% CO_2 for 2 h to remove adherent cells. For immunophenotyping experiments, PBL were incubated for 30 min on ice with primarily conjugated antibodies and washed twice. Mouse monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or Peridinin Chlorophyll Protein (Per CP), reacting with surface markers CD3, CD4, CD8, CD14, CD20, CD16/56, CD57, CD95, CD45RO, and CD45RA, were purchased from Becton-Dickinson (Mountain View, CA). Fluorescence was examined on a FACScan (Becton-Dickinson), and data analysis was performed using Cell Quest software (Becton-Dickinson) [19,20].

T cell activation assay

T cell function/CD3 signaling was assessed at the single cell level by measuring upregulation of T cell surface activation markers CD69 and CD25 following an 18-h exposure of PBL to plate-bound anti-CD3 (biotinylated anti-CD3, Pharmingen, San Diego, CA). To assess basal receptor expression, cells were also tested in uncoated wells. Following the 18-h incubation, cells were washed and stained with a combination of antibodies, including CD4-FITC/CD69-PE, CD8-FITC/CD69-PE, CD4-FITC/CD25-PE, and CD8-FITC/CD25-PE. Mean fluorescence intensity (MFI) was calculated and compared with control values as previously described [21].

Cytokine mRNA levels

Th1 (IL-2 and IFN-γ) and Th2 (IL-4 and IL-10) cytokine mRNA levels were determined by Q-PCR. PBL ($0.5-1.0 \times 10^6$) maintained in complete RPMI media (10% fetal calf serum, penicillin 1000 U/ml, streptomycin 1000 U/ml, and glutamine 20 mM) were placed in Costar plates with medium alone or stimulated with Phorbol 12-Myristate 13 Acetate (75 ng/ml, Sigma, St. Louis, MO) plus ionomycin (1 μM, Sigma). Following an 18-h incubation, cells were lysed and RNA isolated with the Qiagen RNeasy mini kit (Qiagen, Valencia, CA). cDNA was generated using Gibco BRL Superscript First Strand Synthesis system (Invitrogen Corp., Carlsbad, CA). Q-PCR was performed according to manufacturer's instructions as previously described [22]. Briefly, 2.5 μl of cDNA (1—2 ng) was added to an aliquot of 22.5 μl of the stock buffer, bringing the mixed solution to a final concentration of 1× TaqMan mixture (5.5 mmol/l $MgCl_2$, 200 μmol/l dATP/dCTP/dGTP, 400 μmol/l dUTP, 400 nmol/l of primers, 100 nmol/l probe, 0.01 U/μl AmpErase, and 0.025 U/μl AmpliTaq Gold DNA polymerase). 2× TaqMan PCR Master Mix was used to make the stock buffer (PE Biosystems). 25 μl (1—2 ng cDNA in 1× TaqMan mix) was transferred to a 96-well plate. PCR was performed at 50°C for 2 min and at 95°C for 10 min and run for 40 cycles at 95°C for 15 s and at 60°C for 1 min on the ABI Prism 7700 Detection System (Applied Biosystems, Foster City, CA). As a control for RNA input and RT efficiency, 18S rRNA was quantified in each sample with Q-PCR by using a kit purchased from Applied Biosystems.

Quantitative real-time PCR data analysis and molecular confirmation of AT

Data from Q-PCR experiments were analyzed using the comparative C_T method for multiplex reactions, as outlined in User Bulletin No 2 of Applied Biosystems (Foster City, CA). The $2^{-\Delta\Delta CT}$ was used as described previously [22]. The value of each sample was an average of three independent Q-PCR measurements.

Molecular studies supportive of the A-T diagnosis, including colony survival assay, western blot, and DNA sequencing, were performed as previously published [18].

Statistical analysis

Statistical analysis was performed using unpaired Student's *t* test. A probability value of $P < 0.05$ was considered significant.

Results

Patient characteristics

Four A-T subjects were studied; two of these (UPN3 and UPN4) were siblings (Table 1). All subjects were diagnosed in