Selective Deficiency of CD4+/CD45RA+ Lymphocytes in Patients with Ataxia-Telangiectasia

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Several immunological abnormalities have been observed in ataxia-telangiectasia (AT), the most consistent being defects of immunoglobulin isotypes, decreased T-cell numbers, and reduced proliferative responses to mitogens. We examined the distribution of T lymphocytes expressing distinctive surface Ag characteristic of "naive" (CD45RA+) and "memory" (CD29+, CD45RO+) T cells, in both CD4+ and CD8+ (bright and dim) lymphocytes from 13 AT patients, compared with healthy age-matched controls. We found that, irrespective of age, patients with AT had a severe deficiency of CD4+/CD45RA+ lymphocytes. This decrease accounted for the reduction of total CD4+ cells, since the absolute numbers of memory CD4+ cells were not significantly different in AT and in controls. Functional tests revealed poor proliferative responses to phytohemagglutinin and normal responses to soluble Ag (tetanus toxoid) in AT patients. These data fit with the distribution of naive and memory cells, which are known to respond predominantly to mitogens or to recall Ag, respectively. CD45RA molecules were normally expressed on CD8+ lymphocytes. This rules out a generalized defect of regulation or differential splicing as the cause of defective expression of CD45RA on CD4+ cells. The selective deficiency of CD4+/CD45RA+ may provide a cellular basis for some functional T-cell abnormalities of AT patients. Furthermore, it might practically serve for an early, or even prenatal, diagnosis of this disease.

KEY WORDS: Ataxia-telangiectasia; CD45RA+ cells; mitogen and antigen T-cell responses.

INTRODUCTION

Ataxia-telangiectasia (AT) is a rare multisystemic disease affecting the cerebellum, small vessel walls, and several glands including the thymus and gonads (1, 2). Progeric changes are a prominent feature of AT patients, and severe defects of the immune system are also present. These affect both B- and T-cell lineages, resulting in deficiency of IgA, IgG2, IgG4, and IgE (3) and in numerical and functional defects of T lymphocytes (4, 5). The cause of the disease is thought to lie in a defective DNA recombination system, which operates both in the repair of radiogenic DNA damage and in somatic rearrangements involved in the assembly of immunoglobulin and T-cell receptor (TCR) genes (6, 7), perhaps similar to the defect of SCID mice (8).

CD4+ (helper/inducer) T lymphocytes represent a heterogeneous subset, a small portion of which probably exerts true helper activity on B-cell differentiation. Both functional and phenotypic analyses have been used in order to identify distinct subpopulations among CD4+ or CD8+ cells. Two mutually exclusive subsets are identified by the expression of different isoforms of the CD45 surface molecule, also termed leukocyte common antigen (LCA) T-200 (9, 10). These two isoforms, CD45R0 and CD45RA, of relative molecular masses (Mr) of 180–220 kD, respectively, arise from the differential usage of three exons of a single gene (11).

Lymphocytes with the CD4+/CD45R0+ phenotype coexpress other molecules, which are either absent or found at a low density in the reciprocal CD4+/CD45RA+ population. Among these are CD29 (β1 chain of the very late antigen group of integrins), CD44 (also known as Pgp-1), CD2, and CD58 (LFA3) (12, 13).
The subset expressing CD29 and CD45R0 was thought to identify helper cells for B lymphocytes (14, 15), whereas cells expressing CD45RA were thought to contain suppressor-inducer activity for CD8+ lymphocytes (16). These two CD4+ subsets have subsequently been demonstrated to represent antigen inexperienced or naive (CD45RA) and memory (CD45R0) cells, respectively (17). CD45RA cells respond strongly to mitogens, but upon stimulation they lose this marker and acquire CD45R0 (18). Only CD45R0+ memory cells respond in culture to soluble antigens, such as tetanus toxoid, and they have been reported to be the main producers of γ-interferon (γIFN) (12, 19).

AT patients show abnormal distributions of T-cell subsets in peripheral blood, such as reduced proportions of CD4+ cells (4, 5) and of cells bearing the α/β receptor, with an increased ratio of α/β to α/β T lymphocytes (20). A number of functional T-cell effects have also been described in AT patients, including reduced proliferative responses to mitogens (21) and helper activity for immunoglobulin synthesis (4, 5) as well as impaired production of interleukin-2 (IL2) and γIFN (22).

To investigate further the cellular basis of these abnormalities, we characterized the CD4+ subsets in patients with AT by phenotypical and functional studies, including in vitro responses to mitogens and soluble antigens.

MATERIALS AND METHODS

Patients and Controls

We studied 13 patients with AT. The diagnosis was based on the characteristic clinical symptoms, increased levels of α-1-fetoprotein, and demonstration of defective radiation-induced DNA repair (1). Ten of the patients received i.v. γ-globulin as treatment for their hypogammaglobulinemia or IgG subclass defect (two and eight cases, respectively). None of the patients had acute infections or other diseases at the time of the study.

Twenty-one healthy young adults and seven children, selected for age, were tested as normal controls.

Cell Cultures

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by Ficoll/Hypaque (Lymphoprep, Pharmacia, Milan, Italy) density centrifugation, washed three times in phosphate-buffered saline (PBS), and suspended in 10% fetal calf serum (FCS)-supplemented RPMI 1640 (GIBCO, Grand Island, NY). Cell viability (>98%) was determined by trypan blue uptake. Cells were stimulated for 3 days with phytohemagglutinin (PHA-M; Wellcome, UK) at a 1/100 final dilution of stock or for 7 days with tetanus toxoid (TT; Sclavo, Siena, Italy) used at 2.5 Lf/ml (20.5 μg/ml). Doses were selected after preliminary experiments showing the optimal concentration for normal lymphocyte stimulation. Cells were cultured at 1 × 10^6/ml in triplicates in sterile 96-well culture plates (Linbro) in RPMI 1640 supplemented with 2 mM l-glutamine and 10% FCS for experiments with PHA, and 10% autologous serum in TT stimulations, and 100 U/ml penicillin/streptomycin, in a 95% humidified atmosphere with 5% CO₂ at 37°C. Cultures were pulsed for 8 hr with 1 μCi/well of [methyl-3H]thymidine (Amersham) and harvested onto glass-fiber filters. Incorporation was determined by liquid scintillation counting (Packard Instruments, Warerville, IL); data are expressed as mean ± SE cpm of triplicate cultures.

Immunofluorescence (IF) Analysis

Blood samples were selected in tubes containing 1.5 mg of EDTA-K2/ml of blood; 100 μl of well-mixed whole blood was delivered into the test tubes and placed in an ice-water bath. The appropriate amount of monoclonal antibody (10 μl for Ortho-mune, 20 μl for Becton Dickinson, 5 μl for Coulter mAbs) was delivered into each tube. The following monoclonal antibodies (mAbs) were used: phycoerythrin (PE)-conjugated anti-CD29 (4B4), PE-conjugated anti-CD45RA (2H4) (Coulter Immunology, Hialeah, FL), unconjugated anti-CD45R0 (UCHL1) (a gift from P. C. L. Beverley, UCH, London), fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (OKT4) (Ortho Diagnostic System, Raritan, NJ), FITC-conjugated anti-CD8 (Leu 2a), FITC-conjugated anti-CD45RA (Leu18), PE-conjugated anti-CD4 (Leu3a) (Becton Dickinson Immunocytometry System, Mountain View, CA), and unconjugated anti-CD58 (LFA-3) (a gift from T. A. Springer, Harvard Medical School, Boston, MA).

For double fluorescence analysis we added in the same tube OKT4 FITC-conjugated and 4B4 or 2H4 PE-conjugated mAbs. After a gentle mix the samples were incubated in an ice bath for 30 min. Following incubation, the samples were removed, and 2 ml of lysing solution (Ortho) was added to