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Deficiencies in CD4$^+$ and CD8$^+$ T cell subsets in ataxia telangiectasia

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SUMMARY

Chronic sinopulmonary infections that are associated with immunodeficiency are one of the leading causes of death in the multi-systemic disease ataxia telangiectasia (AT). Immunological investigations of AT patients revealed a broad spectrum of defects in the humoral and the cellular immune system. Based on their important role in host defence the aim of our study was an extensive analysis of cell distribution and function of CD4$^+$ and CD8$^+$ T lymphocytes and NK cells. We found that naive (CD45RA$^+$) CD4$^+$ lymphocytes, as well as CD8$^+$/CD45RA$^+$ lymphocytes, are decreased, whereas NK cells (CD3$^-$/CD16$^+$CD56$^+$) are significantly elevated in AT patients. In our culture system proliferation and cytokine production was normal in purified memory (CD45RO$^+$) lymphocytes after stimulation with phorbol-12,13-dibutyrate (PBu_2) and after PHA activation, indicating that differences in proliferation and cytokine production are due solely to reduced numbers of CD45RA$^+$ lymphocytes. However, activation, and especially intracellular interferon production of AT lymphocytes, seem to follow different kinetics compared to controls. In contrast to polyclonal activation, stimulation via the T cell receptor results consistently in a reduced immune response. Taken together, our results suggest that deficiency of immunocompetent cells and an intrinsic immune activation defect are responsible for the immunodeficiency in AT.

Keywords ataxia telangiectasia CD4$^+$ cells CD45RA$^+$ (naive) lymphocytes CD8$^+$ cells T cell activation

INTRODUCTION

The syndrome of ataxia telangiectasia (AT) is caused by a single autosomal recessive genetic defect on chromosome 11q22–23, and includes a variety of apparently unrelated features such as progressive cerebellar ataxia, telangiectasia, thymic dystrophia with immunodeficiency, chromosomal instability, radiosensitivity and cancer susceptibility [1,2]. This pleiotropic phenotype is attributed to the mutated ATM gene on chromosome 11q22–23 and its gene product which belongs to a family of proteins involved in cell cycle control, DNA repair and signal transduction [3–6].

Most patients suffer from progressive respiratory infections that lead frequently to pulmonary insufficiency and death [7]. Laboratory findings indicate a variable immunodeficiency in both the humoral and cellular immune system. Thus, decreased IgE, IgA and IgG_2-subclass production, defective B cell receptor signalling, reduced response to mitogens, impaired capacity to produce cytotoxic T lymphocytes and reduction of peripheral lymphocytes have been described by several authors [8–13]. Paganelli *et al*. [14] found a selective deficiency of naive (CD45RA$^+$) CD4$^+$ lymphocytes in AT patients while no changes in the number of memory (CD45RO$^+$) cells have been observed. Naive and memory cells represent different stages in T cell development of T-helper cells (CD4$^+$) and cytotoxic T-cells (CD8$^+$) and have different activation, proliferation and cytokine patterns [15]. Changes in the naive/memory cell subsets may help us understand immunodeficiency in AT.

Experimental data from ataxia telangiectasia mutated $(Atm)^{-/-}$ mice confirm the pleiotropic defects in AT patients. Lymphoid tissues from $Atm^{-/-}$ mice are generally smaller when compared with control mice, and there is inappropriate T cell development and function [16–19]. Nevertheless, unlike in humans, investigation of peripheral T lymphocytes in $Atm^{-/-}$ mice shows cell loss in both the CD4$^+$ and CD8$^+$ populations [18]. Recent investigations in $Atm^{-/-}$ mice indicate that the T cell-dependent immune response is secondary to reduced T cell numbers [20].

Although lymphocyte subsets and T cell function have been studied intensively in AT, there are still conflicting reports about the number and integrity of immunocompetent cells. This prompted us to a more extensive analysis of cell distribution and function of CD4$^+$ and CD8$^+$ T lymphocytes, especially of CD45RA$^+$, CD45RO$^+$ cells and NK cells.

Our analysis demonstrated significantly diminished naive CD4$^+$ and CD8$^+$ T lymphocytes and elevated levels of NK cells in

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AT patients. Additionally we used long-term (72 h) and short-term cell culture (5 h) to investigate functionality and cell activation. We found that stimulation via the T cell receptor results in a reduced immune response whereas no differences were found after polyclonal activation of memory T cells. However, activation and cytokine production of AT T lymphocytes differ from controls and seem to follow a different kinetic.

MATERIALS AND METHODS

Subjects

Nineteen patients with AT (median age 13·0 years; range 4–27 years; sex distribution M : F, 10 : 9) were studied. Diagnosis was established in accordance with the WHO recommendations [21]. All patients showed increased levels of α-fetoprotein and atypical G2 arrest measured by flow cytometry (data not shown). Ethylene diamine tetra-acetic acid (EDTA) anticoagulated blood for lymphocyte phenotyping and heparinized samples for cell culturing experiments were collected from patients and aged matched healthy controls. The study was approved by the local Human Committee of Ethics and informed consent was obtained from patients and parents before entering the study.

Lymphocyte phenotyping

Whole blood samples containing EDTA were stained with the following monoclonal antibodies (MoAb) directly labelled either with fluorescein-isothiocyanate (FITC), phycoerythrin (PE) or PerCP: UCHT-1 (CD3), B9·11 (CD8), J4·119 (CD19), ALB11 (CD45RA), UCHL-1 (CD45RO) from Coulter-Immunotech (Marseille, France), RPA-T4 (CD4), 3GA (CD16) from PharMingen (San Diego, CA, USA) and MY31 (CD56) from Becton Dickinson (San Jose, CA, USA). After staining, erythrocytes were lysed (FACS-lysing solution, Becton Dickinson), washed with PBS/0·1% Na azide and fixed with 1% paraformaldehyde (PFA). Cells were incubated for 15 min at room temperature (RT) and 10 000 events were analysed with a flow cytometer and data analysis was performed with Lysis II software (FACScan, Becton Dickinson).

Cell culture

Cells were cultured in 96-well flat-bottom plates in RPMI 1640 supplemented with 10% FCS, 1% penicillin/streptomycin, 1% HEPES, 2% glutamine and 0·2% gentamicin at a concentration of 1×10^6 cells/ml. For induction of cell proliferation and cytokine production, lymphocytes were incubated 72 h in the presence of phythaemagglutinine (PHA) (1 μg/ml), IL-2 (100 U/ml) or phorbol-12,13-dibutyrate (PBu_2, 1 ng/ml) in conjunction with ionomycin (0·5 μg/ml). Alternatively, cells were stimulated with anti-T cell receptor (TCR) MoAb BMA031 coated on beads (125 ng BMA031/$2{\cdot}5 \times 10^6$ Dynabeads M450) and co-stimulated with anti-CD28 MoAb (BMW828, 2·5 μg/ml).

For intracellular staining of IFN-γ, cells were stimulated exclusively with PBu_2 (1 ng/ml) together with ionomycin (0·5 μg/ml) for 5 h. Cytokine secretion was blocked by monensin (2·5 μg/ml).

Intracellular cytokine staining

Cultured cells were washed twice in PBS/0·1% Na azide and surface antigens were stained as mentioned above. For intracellular staining, cells were fixed in cold PBS containing 4% PFA for 10 min. After two further washes in PBS/0·1% Na azide, cells were resuspended in 100 μl PBS containing 0·1% saponin and 50 μl Cytotect (Biotest) was added. Cells were incubated for 10 min at RT, spun down and FITC-labelled IFN-γ-specific MoAb (Hölzel Diagnostika GmbH, Cologne, Germany) diluted in PBS with 0·1% saponin added at a concentration of 1 μg/ml and incubated for 30 min at RT. As a last step before FACS-analysis, cells were washed in PBS/0·1% Na azide.

Quantification of intracellular IFN-γ levels

Quantitative determination of intracellular IFN-γ was accomplished by the Quantum Simply Cellular® kit, Anti-human IFN-γ FITC (Hölzel Diagnostika GmbH, Cologne, Germany). Microbeads labelled with different levels of interferon-γ FITC MoAb were used to calibrate the system where the cells labelled with IFN-γ antibodies could be quantified by their Antigen Binding Capacity (ABC); 5000–10 000 cells were computed in list mode and analysed using the QuickCal® research software (QuickCal®, Flow Cytometry Standard Corp., San Juan, CA, USA).

Purification of $CD45RO^+$ cells

Peripheral mononuclear blood lymphocytes from AT patients and healthy controls were obtained by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). To remove most of the adherent cells, cell suspensions were first incubated for 1 h at 37°C in plastic Petri dishes, and then T cells were enriched by rosetting with sheep red blood cells. From these T cells, $CD45RO^+$ subsets were purified by using negative selection with anti-CD45RA-specific mouse MoAb and goat antimouse Ig-coated magnetic beads (Dynal GmbH, Hamburg, Germany). This was performed according to the manufacturer's instructions and the purity (80–90%) of the subsets was tested by flow cytometry.

Cell proliferation

For measurement of DNA synthesis cells, were incubated for 72 h under conditions described above. BrdU (5-bromo-2′-deoxyuridine) incorporation was detected by a colourimetic cell proliferation ELISA (BrdU-ELISA, Boehringer Mannheim) after 18 h incubation.

ELISA

After a culture period of 48 h, cell culture supernatants were harvested. IL-2 and IFN-γ were detected by commercially available ELISA-kits (ELISA, Genzyme, Cambridge, MA, USA). Measurement was performed according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using either unpaired Student's *t*-test or, if the data were not normally distributed, the Mann–Whitney *U*-test (SPSS Inc., Chicago, IL, USA). In both tests, a probability value of $P < 0{\cdot}05$ was considered significant.

RESULTS

Cell differentiation

Patients with AT have significant alterations in their lymphocyte phenotype (Table 1). Both the $CD8^+/CD45RA^+$ and $CD4^+/CD45RA^+$ T cells were diminished to the same extent with no difference from controls in the number of $CD8^+/CD45RO^+$ or $CD4^+/CD45RO^+$ cells.

Table 1. Lymphocyte subsets of patients with ataxia telangiectasia and controls

Population (subpopulations)	*n*	Controls cells/μl	*n*	Patients cells/μl	*P* <
$CD3^+$	20	1509 (786–6663)	19	845 (624–1926)	0·01
$CD3^+CD4^+$	20	992 (484–4640)	19	473 (99–1448)	0·01
$CD3^+CD4^+CD45RA^+$	20	490 (178–3832)	19	61 (29–873)	0·01
$CD3^+CD4^+CD45RO^+$	20	411 (78–818)	19	428 (147–2266)	n.s.
$CD3^+CD8^+$	20	652 (216–1782)	19	350 (167–724)	0·005
$CD3^+CD8^+CD45RA^+$	13	459 (264–1628)	12	115 (72–262)	0·01
$CD3^+CD8^+CD45RO^+$	13	231 (116–755)	12	267 (123–644)	n.s.
$CD3^-CD16^+CD56^+$	20	217 (43–1245)	19	664 (271–1469)	0·001

Results represent median and range of cells per microlitre. *P*-value based on Mann–Witney *U*-test compared with controls. $P > 0{\cdot}05$, n.s., not significant.

$CD8^+$ lymphocytes can be divided into $CD8^{bright}$ and $CD8^{dim}$ cells. The former are exclusively $CD3^+$ T cells, and the loss of naive cells is based mainly in this population. Most $CD8^{dim}$ cells belong to the NK cell population ($CD3^-/CD56^+CD16^+$) which expresses exclusively the $CD45RA^+$ phenotype; this could give the misleading impression of a 'well-balanced' ratio of CD45RA:CD45RO lymphocytes because the number of NK cells is significantly elevated in AT compared to controls (Table 1).

Lymphocyte proliferation

Proliferation of peripheral lymphocytes was studied in long-term culture (72 h) in the presence of PHA, phorbol-12, 13-dibutyrate (PBu_2) in conjunction with ionomycin or anti-TCR MoAb-coated beads with and without CD28 co-stimulation. Similar to previous experiments, peripheral blood mononuclear cells (PBMC) showed reduced proliferative activity and impaired IL-2 and IFN-γ production (Fig. 1a–c).

To answer the question of whether the loss of naive cells in $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cell populations was responsible for the reduced cell activity, we purified $CD45RO^+$ cells from patients and controls and stimulated them in the same manner. Indeed, there was no difference in cell proliferation and in cytokine production of negative selected $CD45RO^+$ cells between AT lymphocytes and controls, either after stimulation with PBu_2/ionomycin or PHA stimulation (Fig. 1d–f). These findings indicate that the decreased immune response in AT is due to the loss of immunocompetent cells. Nevertheless, as found in PBMC, isolated $CD45RO^+$ lymphocytes still exhibited decreased proliferation, as well as reduced cytokine production, to T cell receptor dependent activation compared to controls (Fig. 1d–f).

Enhancement of T cell receptor dependent cell activation

To enhance decreased IFN-γ production of total AT lymphocytes, we added IL-2 to PHA or TCR/CD28-stimulated PBMC. Co-stimulation with IL-2 had no effect on IFN-γ production in control cells treated with PHA or stimulated via the TCR (Fig. 1g). In contrast, IL-2 addition resulted in a distinct modification of IFN-γ response in PHA-stimulated cells, whereas stimulation of the TCR/CD28 pathway had very little effect on patient lymphocytes.

IFN-γ producing cells

Given the reduced numbers of $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells and the increased number of NK cells ($CD3^-/CD56^+CD16^+$), we tested the function of these lymphocyte populations by measuring IFN-γ production. Cells were stimulated for 5 h (short-term culture) with PBu_2/ionomycin and IFN-γ production detected by intracellular flow cytometry. To confirm that cells were activated, the expression of the activation inducer molecule CD69 was measured simultaneously.

In all investigated populations the mean percentage of $CD69^+$ cells after stimulation with PBu_2/ionomycin was lower in patients than in controls, reflecting impaired activation of AT lymphocytes (Fig. 2a–c). In relation to the total number of stimulated AT cells, about 50% less $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cells were activated compared to controls. In contrast, the total number of activated CD69 expressing NK cells ($CD3^-/CD16^+CD56^+$) was higher in patients than in controls due to the increased number of total NK cells (data not shown).

Analysis of IFN-γ expressing $CD3^+/CD4^+$ and $CD3^+/CD8^+$ cells revealed a higher percentage of IFN-γ producing lymphocytes in patients, whereas in the NK cell population the percentage of activated cells is reduced compared to controls (Fig. 2d–f). Nevertheless, when related to the total number of stimulated PBMC, IFN-γ producing cells from patients were in the range of controls in all investigated cell populations (Table 2). Our findings indicate that although patients show less activated lymphocytes, the total number of IFN-γ^+ cells is similar in patients and controls.

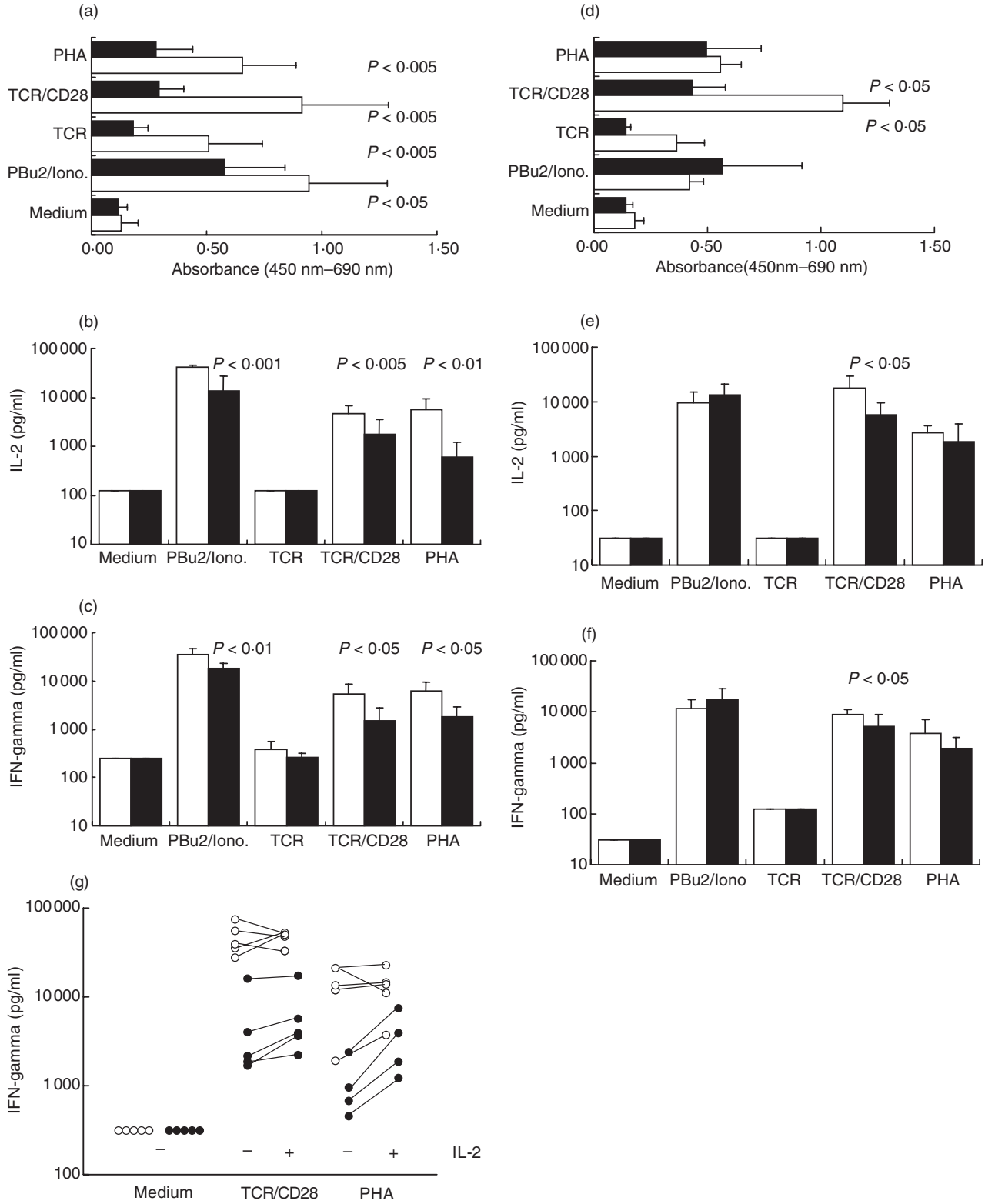

Fig. 1. Function of peripheral blood mononuclear cells (PBMC) and CD45RO$^+$lymphocytes. Figure shows stimulation of PBMC (a–c) and separated CD45RO$^+$ lymphocytes (d–f) derived from AT patients (filled bars, $n = 8$) and controls (open bars, $n = 10$). Cells were stimulated with phorbol-12,13-dibutyrate (PBu_2) and ionomycin, PHA and via the T cell receptor with and without CD28 co-stimulation. Proliferation (a + d), IL-2 production (b + e) and IFN-γ production (d + f) were detected by BrdU incorporation or by ELISA, respectively. To investigate whether IFN-γ production could be enhanced, cells were stimulated additionally with IL-2 (g). Results are shown as mean ± s.d. *P*-values based on Student's *t*-test.

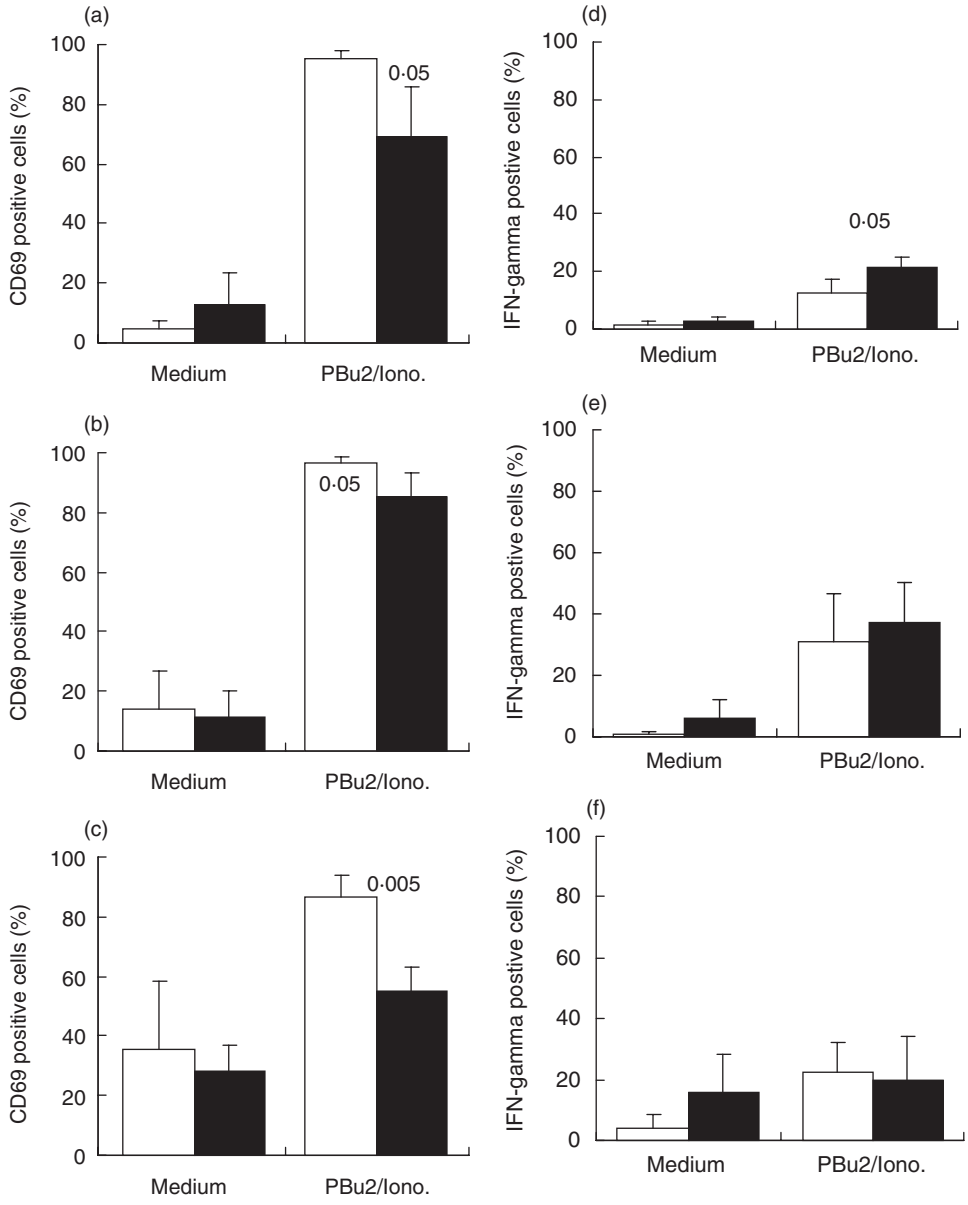

Fig. 2. Proportion of activated lymphocytes ($CD69^+$) in $CD3^+/CD4^+$ T cells (a), $CD3^+/CD8^+$ T cells (b) and NK cells (c) and analysis of intracellular IFN-γ expression in $CD3^+/CD4^+$ T cells (d), $CD3^+/CD8^+$ T cells (e) and NK cells (f). Lymphocytes from AT patients (filled bars, $n = 5$) and controls (open bars, $n = 8$) were stimulated with phorbol-12,13-dibutyrate (PBu_2) and ionomycin for 5 h. Cytokine secretion was inhibited by monensin. Results are shown as mean ± s.d. *P*-values based on Student's *t*-test.

Intracellular IFN-γ quantification

We analysed the amount of IFN-γ in $CD3^+/CD4^+$, $CD3^+/CD8^+$ and NK-cells ($CD3^-/CD16^+CD56^+$) by an intracellular cytokine quantification assay. As shown in Fig. 3a–c, cytokine production in AT was decreased in the $CD3^+/CD4^+$ as well as in the $CD3^+/CD8^+$ cell population. In NK cells, the amount of intracellular IFN-γ was not different from controls. Summarized, in AT the percentage of activated lymphocytes was diminished and the level of produced IFN-γ per cell was reduced in $CD3^+/CD4^+$ and in $CD3^+/CD8^+$ lymphocytes.

IFN-γ production by CD45RO⁺ cells

To confirm that the defective activation of AT $CD3^+/CD4^+$ and $CD3^+/CD8^+$ lymphocytes was not an artefact of $CD45RA^+$ cells, we tested activation and intracellular IFN-γ levels of $CD45RO^+$ cells diretly from AT patients and controls after 5 h PBu_2 stimulation. As $CD4^+$ and $CD8^+$ lymphocytes, the $CD45RO^+$ cell population exhibited a reduced number of activated $CD69^+$ cells and intracellular IFN-γ levels were reduced (data not shown). This points to a general activation deficiency regardless of the T cell type in AT.

DISCUSSION

Sinopulmonary infection leading to irreversible pulmonary damage is the major cause of premature death in AT [22]. Both the humoral and cellular arms of the immune system are likely to be involved in this process [5]. In accordance with previous

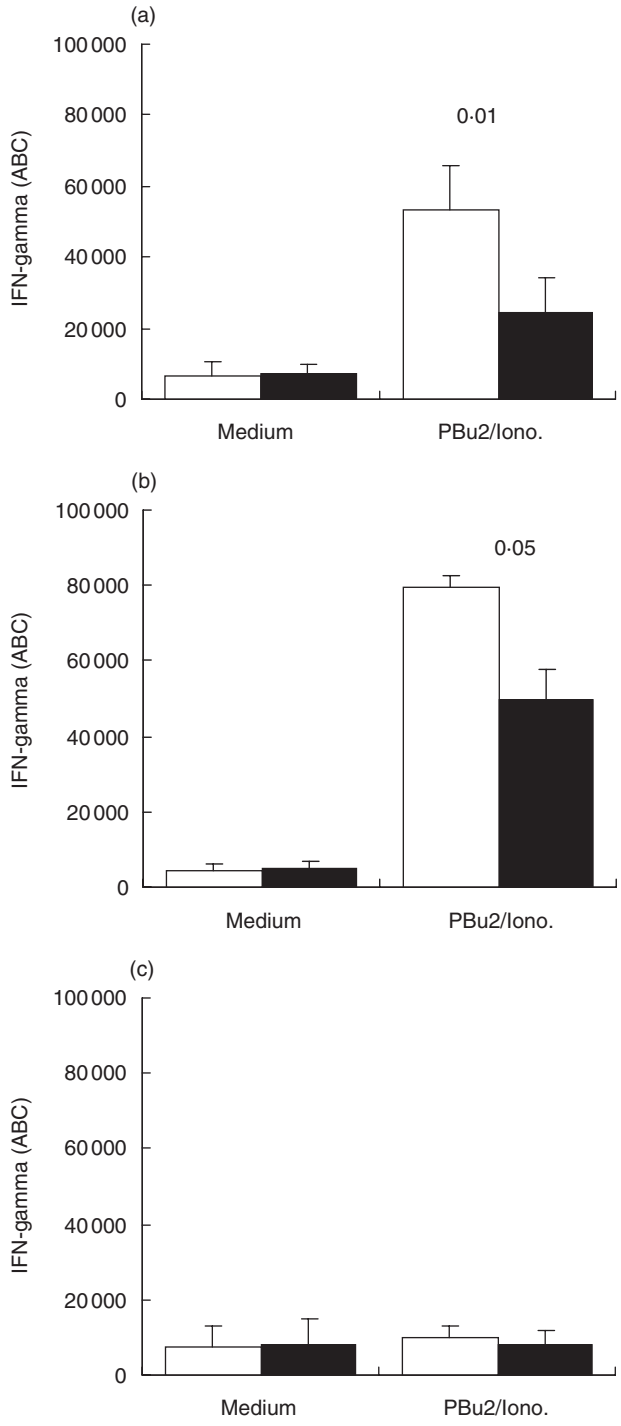

Fig. 3. Intracellular IFN-γ quantification. Intracellular IFN-γ level of PBMC derived from AT patients (filled bars, $n = 5$) and controls (open bars, $n = 8$) were investigated by intracellular flow cytometry. Amount of IFN-$\gamma$was quantified by antigen binding capacity (ABC). Therefore, mean fluorescence of microbeads labelled with different levels of IFN-γ FITC MoAb were detected and calibration plot and intracellular IFN-γ levels (a–c) were calculated using the QuickCal® research software. Lymphocytes were differentiated in $CD3^+/CD4^+$ cells (a), $CD3^+/CD8^+$ cells (b) and $CD3^-/CD16^+CD56^+$ cells (c). Cells were stimulated with phorbol-12,13-dibutyrate (PBu_2) and ionomycin for 5 h and cytokine secretion was inhibited by monensin. Results are shown as mean ± s.d. *P*-value based on Student's *t*-test.

studies, our analysis revealed that T cell loss and defective T cell function are important features of the immunological disorder in AT [23]. In addition, we demonstrate that T cell loss is due not only to a decrease of naive ($CD45RA^+$) $CD3^+/CD4^+$ lymphocytes but also to a loss of naive ($CD45RA^+$) $CD3^+/CD8^+$ lymphocytes. As in the $CD3^+/CD4^+$ population, lymphopenia in $CD3^+/CD8^+$ cells was found only in the $CD45RA^+$ population, whereas the total number of $CD45RO^+$ lymphocytes did not differ from controls. These findings are in contrast to Paganelli *et al*. [14], who reported a selective deficiency of circulating $CD3^+/CD4^+$ lymphocytes due to a reduction of naive ($CD45RA^+$) cells. This discrepancy appears to be related to elevated numbers of $CD3^-8^{dim}$ NK cells, which were described as $CD3^+/CD8^+$ cells and not recognized as NK cells. Our findings agree with Barlow *et al*. [18], who showed that T lymphocytes from $Atm^{-/-}$ mice displayed a reduction of both $CD3^+/CD4^+$ and $CD3^+/CD8^+$ double positive cells in lymphoid tissues when compared to control mice. These results show that the defect in T cell maturation is not restricted to the T helper/inducer cells but also affects the T killer/suppressor cell population; we can speculate that the expansion in NK cells ($CD3^-/CD16^+CD56^+$) in AT patients compensates to some extent for the immunodeficiency.

The reason for the lymphopenia in AT is currently unknown, but is caused probably by the abnormal development of the thymus [8,24,25]. Dysplastic changes in the thymus could reduce the release of naive cells, with an accumulation of memory cells in the periphery [14,26]. Alternatively, as proposed for a murine model by Bell and Sparshott [27], the naive cell loss may be due to a failure of conversion of CD45RO back to the CD45RA phenotype.

Another possibility is that enhanced levels of oxidative stress, as found in lymphocytes in $Atm^{-/-}$ mice [16,18], lead to premature cell death. Lahdenpohja *et al*. [28] showed that naive T cells are more sensitive to oxidative stress than memory cells. The premature ageing associated with AT may also be related to increased oxidative stress and NK cell numbers [29].

Several authors reported impaired function of AT lymphocytes. This includes defects of T helper/inducer lymphocytes [10,26,30] and of T suppressor/killer cells [1,10]. To prove the hypothesis that the reduced number of lymphocytes is responsible for the defective immune response in AT, we compared functionality of AT lymphocytes to control cells with the same phenotype. Because of low levels of $CD45RA^+$ lymphocytes in AT, memory cells were chosen. Cell proliferation, IFN-γ and IL-2 production were normal in AT $CD45RO^+$ lymphocytes after stimulation with phorbol-12,13-dibutyrate (PBu_2) and ionomycin or PHA, indicating that the functional defect is due to loss of naive lymphocytes. In the same cell system, stimulation via the T cell receptor results in significantly reduced proliferation and cytokine production when compared to controls. This is in keeping with previous reports of defective calcium-dependent signal transduction in T cells, and defective signalling via the B cell antigen receptor in AT [13,31].

The fundamental cause of this defective signalling in AT is still not understood, although defective rearrangement of the TCR itself has been suggested [32–34]. In an attempt to quantify cytokine production in AT, we chose IFN-γ expressed in stimulated $CD4^+$, $CD8^+$ and NK cells in short-term cultures, using CD69 as a marker for cell activation. Interestingly, the percentage of activated $CD69^+$ cells was decreased in all AT lymphocyte populations, including the NK cells. Since CD69 is up-regulated early

Table 2. Total number of IFN-γ producing $CD4^+/CD3^+$, $CD3^+/CD8^+$ and $CD3^-/CD16^+$ + $CD56^+$ cells from AT patients controls are shown

	Controls (cells/μl)		Patients (cells/μl)	
	Medium	PBu_2/Iono	Medium	PBu_2/Iono
$CD3^+CD4^+$	$5{\cdot}9 \times 10^3$ ($0{\cdot}2$–$2{\cdot}6 \times 10^4$)	$4{\cdot}7 \times 10^4$ ($0{\cdot}2$–$1{\cdot}3 \times 10^5$)	$6{\cdot}3 \times 10^3$ ($0{\cdot}3$–$1{\cdot}2 \times 10^4$)	$4{\cdot}5 \times 10^4$ ($3{\cdot}1$–$5{\cdot}8 \times 10^4$)
$CD3^+CD8^+$	$1{\cdot}4 \times 10^3$ ($0{\cdot}01$–$1{\cdot}0 \times 10^4$)	$7{\cdot}6 \times 10^4$ ($0{\cdot}1$–$2{\cdot}9 \times 10^5$)	$5{\cdot}4 \times 10^3$ ($0{\cdot}1$–$1{\cdot}6 \times 10^4$)	$5{\cdot}5 \times 10^4$ ($0{\cdot}2$–$1{\cdot}0 \times 10^5$)
$CD3^-CD16^+CD56^+$	$3{\cdot}7 \times 10^3$ ($0{\cdot}01$–$3{\cdot}4 \times 10^4$)	$2{\cdot}8 \times 10^4$ ($0{\cdot}2$–$7{\cdot}9 \times 10^4$)	$1{\cdot}6 \times 10^4$ ($0{\cdot}3$–$4{\cdot}5 \times 10^4$)	$2{\cdot}7 \times 10^4$ ($0{\cdot}8$–$5{\cdot}8 \times 10^4$)

Values represent median and range of cells per microlitre. PBu_2, phorbol-12,13-dibutyrate.

after phorbolester stimulation, this suggests that early activation is incomplete in AT. This finding was supported by a decrease in intracellular IFN-γ in $CD4^+$ and $CD8^+$ T cells while the absolute number of IFN-γ producing cells in both populations was in the normal range. This was also found in $CD45RO^+$, cells showing that the reduction in cell activation is not only restricted to naive lymphocytes. This defect in activation could be enhanced significantly by the addition of IL-2 in PHA stimulated cells.

Lavin and Shiloh [5] attribute a role in signal transduction to the ATM gene product, based on its localization in the cytoplasm. Possibly the ATM protein is of importance in the signal pathway from the cell membrane to the nucleus, since the ATM protein has been localized recently to vesicular structures in the microsome, as well to the nucleus [35,36]. In addition an up-regulation of the ATM protein in proliferating cells points to a role for ATM in more general signalling [37].

A recent study by Rivero-Carmena *et al*. [38] showed that HVS-transformed mature peripheral T lymphocytes from AT patients do not exhibit any activation deficit and that the cells do not have an intrinsic immune functional defect. Although they demonstrated that these transformed T cells retained important features of AT cells (i.e. radiosensitivity), the transformed cell lines may be intrinsically activated and may not be a suitable test system for AT.

In conclusion, our results suggest a defect in the early activation of lymphocytes in AT, which may be the underlying cause of the reduced numbers of naive T lymphocytes and the apparent compensating increase in NK cells.

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