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The efficacy of combined therapy of arsenic trioxide and alpha interferon in human T-cell leukemia virus type-1-infected squirrel monkeys (*Saimiri sciureus*)

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Abstract

Human T-cell lymphotropic virus type 1 (HTLV-1)-associated adult T-cell leukemia/lymphoma (ATLL) has a poor prognosis owing to its intrinsic resistance to chemotherapy. Although zidovudine (AZT) and alpha interferon (IFN-α) give rise to some response and improve the prognosis of ATLL, alternative therapies are needed. Arsenic trioxide (As_2O_3) has been shown to synergize with IFN-α in arresting cell growth and inducing apoptosis of ATLL cells in vitro. In this study, we evaluated the toxicity and the efficacy of this combined treatment in HTLV-1-infected squirrel monkeys (*Saimiri sciureus*) and HTLV-1 infected cell lines derived therefrom. We first show that treatment with As_2O_3 and IFN-α can induce growth arrest in HTLV-1-transformed monkey T-cell lines in vitro. We then show that treatment of squirrel monkeys with As_2O_3 in vivo is highly toxic at 0.9 or 0.3 mg/day but not at 0.14 mg/day for up to 2 weeks. Although the combination of As_2O_3 and IFN-α did not affect significantly the HTLV-1 proviral load in infected monkeys, it reduced the absolute numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells during treatment, with a significant reduction in the total number of circulating HTLV-1 flower cells in the infected monkeys with chronic ATLL-like disease.

Keywords: Arsenic; Interferon-alpha; HTLV-1; Squirrel monkeys; Treatment; Proviral load; Flower cells

1. Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia/lymphoma (ATLL) (Yoshida et al., 1984). ATLL is an aggressive lymphoid proliferation that was originally described about three decades ago. The serum of ATLL patients contains antibodies to HTLV-1 (Hinuma et al., 1982), and the HTLV-1 provirus is clonally integrated into ATLL cells (Tsukasaki et al., 1997). As the clinical features of ATLL are diverse, four clinical subtypes have been defined: an acute form, a chronic form, a smoldering form and a lymphoma form. Treatment of patients with the chronic and smoldering forms with conventional chemotherapy is associated with the exacerbation of the cell-mediated immune deficiency and with opportunistic infections, with little benefit on survival, if any (Nakada et al., 1987; Shimoyama, 1991). In general, the anti-ATLL treatment has little or no benefit. In particular, it does not prevent the transformation towards aggressive ATLL. However, the combination of zidovudine (AZT) and interferon-α (IFN-α) may increase the immune function (Hermine et al., 1995). Promising results were also

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reported with the use of unlabelled or radiolabelled anti-IL-2R monoclonal antibodies (Waldmann et al., 1993). Nevertheless, new alternatives that would act by different mechanisms, targeting the HTLV-1 or the viral oncoprotein Tax, or secondary genetic events (Tax-dependent or -independent), are needed.

Arsenic trioxide (As_2O_3) is an effective treatment for acute promyelocytic leukemia (Soignet et al., 1998). Bazarbachi et al. (1999) demonstrated that As_2O_3 synergizes with IFN-α to induce cell cycle arrest and apoptosis in HTLV-1-infected and fresh ATLL cells. These chemicals act through two distinct events: a rapid shut-off of the NF-κB pathway induced by arsenic-triggered stabilization of IκBs, and a delayed arsenic/IFN-triggered shut-off of cell cycle-associated genes, secondary to Tax degradation by the proteasome (El-Sabban et al., 2000; Nasr et al., 2003). Furthermore, in a phase II trial of arsenic/IFN-α in seven patients with relapsed or refractory ATLL (four acute and three lymphoma), four patients had a clear initial response (one complete remission and three partial remissions), and one patient is still alive and disease-free at 32 months (Hermine et al., 2004). These encouraging results demonstrate that treatment of ATLL with arsenic and IFN-α is feasible and elicits a clear anti-leukemic effect even in refractory cases. Prevention of ATLL is a difficult goal to attain in HTLV-1-seropositive patients due to the small number of HTLV-1 carriers who develop ATLL, the lack of predictive factors for ATLL and the inevitable costs. It would, however, be interesting to test whether anti-retroviral drugs could reduce the viral load and clonal expansion of infected T-cells before the disease occurs. The HTLV-1 proviral load is thought to be a strong predictor of disease progression (Nagai et al., 1998; Taylor et al., 1999). Indeed, patients with tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) or adult T-cell leukemia have a higher proviral load than asymptomatic HTLV-1 carriers. Thus, strategies to maintain low proviral load in HTLV-1 asymptomatic carriers may prevent disease development.

Animal models of HTLV-1 infection remain crucial to a better understanding of the biology of viral infection, to better describe the associated pathological manifestations, to design therapeutic strategies and to evaluate the efficacy of vaccine combinations. Chronic HTLV-1 infections have been obtained in both rabbit and rat models. Transgenic mice have also been obtained and were found to be useful for demonstrating the presence, within the viral genome, of oncogenic determinants such as Tax (Benvenisty et al., 1992; Furuta et al., 1989; Green et al., 1989; Yamamoto et al., 1993). Unfortunately, these systems either only partially reflect the human pathological manifestations or do not include an essential parameter, i.e. the viral replication. Transgenics and direct HTLV-1 infection of rats elicited various pathological manifestations (thymoma, chronic destructive arthritis) and allowed the evaluation of the immune response (cytotoxic activity, down-regulation of major histocompatibility complex class I), the assessment of therapeutic strategies (vaccination, adoptive transfer) and the testing of various routes of infection (Ibrahim et al., 1994; Kazanji et al., 1997a,b). Although these model systems were instructive, a major limitation is the lack of adequacy between host and virus specificities, especially during the acute phase of ATLL. Besides alternative strategies based on comparative pathogenesis (i.e. simian T-lymphotropic virus in monkeys), a non-human primate model experimentally infected with HTLV-1 might be the closest model for studying HTLV-associated pathogenesis. We have previously shown that squirrel monkeys (*Saimiri sciureus*), New World primates, are susceptible to experimental infection with syngeneic or allogeneic HTLV-1-immortalized cells (Kazanji et al., 1997c, 2000), and that inoculation of HTLV-1-infected cells in these animals leads to a chronic infection. Antibodies against HTLV-1 are detected in experimentally infected monkeys, and HTLV-1 provirus is detectable in their peripheral blood mononuclear cells (PBMCs) by polymerase chain reaction (Kazanji, 2000). Furthermore, as in human infection, HTLV-1 infection of squirrel monkey leads, after extended latent periods, to the continuous expansion of a restricted number of abundant HTLV-1-infected cellular clones (Mortreux et al., 2001). Furthermore, we showed recently that some chronically infected monkeys had high $CD4^+$ T-cell counts concomitantly with an increased total lymphocyte population. In addition, a significant proportion of these lymphocytes was infected, and flower cells were found in the peripheral blood (Debacq et al., 2005). The squirrel monkey thus appears to be a suitable model not only for studying the pathogenesis of HTLV-1 infection but also because it provides a preclinical platform to assess therapeutic intervention. As we cannot evaluate the effect of such therapy in HTLV-1 human asymptomatic carriers, we have studied here the toxicity and the efficacy of a combined therapy with As_2O_3 and IFN-α in HTLV-1-infected squirrel monkeys (*S. sciureus*) and HTLV-1-infected cell lines derived therefrom.

2. Materials and methods

2.1. Drugs

Recombinant IFN-α (Roche Molecular Biochemicals, Basel, Switzerland) and arsenic trioxide (Sigma, l'Isle d'Abeau, France) were used at 100 units/mL and 1 μmol/L, respectively, as previously described (Bazarbachi et al., 1999; Mahieux et al., 2001). Working stocks were diluted in Roswell Park Memorial Institute culture medium (RPMI 1640) (Life Technologies, Gaithersburg, MD), supplemented with 10% heat-inactivated foetal calf serum (FCS; GIBCO-BRL).

2.2. Squirrel monkeys cell lines

EVO/1540 is a previously reported squirrel monkey HTLV-1-transformed T-cell line (Kazanji et al., 1997c). Ssc/EBV is a previously described squirrel monkey B-cell line transformed by Epstein–Barr virus (Kazanji et al., 2000). These cells lines were grown in RPMI-1640, supplemented with 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 10% recombinant interleukin-2 (Roche Molecular Biochemicals, Basel, Switzerland).

2.3. Cell proliferation and viability assay

HTLV-1- or EBV-transformed monkeys cells were cultured in 96-well, flat-bottom microtiter plates (Nunc, Naperville, IL) in quadruplicates. Test drugs were added at the indicated concentrations at the initiation of culture and then were cultured for 60 h. To measure cellular proliferation or viability, a cell proliferation–viability kit (XTT; Roche, Molecular Biochemicals, Basel, Switzerland) was used. In this assay, tetrazolium salt XTT is cleaved to form an orange formazan dye by metabolically active cells (Mahieux et al., 2001). This dye is quantified directly on an enzyme-linked immunosorbent assay reader at 492 nm. Three independent measures were used to calculate means of cellular proliferation and standard error means (S.E.M.). *p*-Values were obtained using the statistical Student's *t*-test.

2.4. Animals and administration of As_2O_3/IFN-α

Eight adult male squirrel monkeys (average body weight of 1 kg) from the primate breeding center of the Pasteur Institute of French Guiana were used. Four monkeys had previously been intravenously inoculated with 5×10^7 monkey HTLV-1-transformed cells, as previously described (Kazanji et al., 1997c), and have been HTLV-1-seropositive for 8 years, as evidenced by a positive anti-HTLV-1 serology in both enzyme-linked immunosorbent assay (Diagnostic Biotechnology Ltd., Singapore) and western blot (HTLV blot 2.3, Diagnostic Biotechnology Ltd., Singapore) techniques.

Under ketamine anesthesia, the monkeys were surgically implanted intraperitoneally with a mini-osmotic pump (Alzet, Cupertino, CA), which was filled under sterile conditions in accordance with the Manufacturer's instructions. In initial experiments, we tested the toxicity of As_2O_3 in squirrel monkeys at two doses, (0.9 and 0.3 mg/day) for 1 month. In the second set of experiments, six monkeys (four HTLV-1-infected and two controls) received a unique dose of 0.14 mg/day (total of 2 mg per monkey) for 2 weeks. A 0.1% As_2O_3 solution (10 mg in 10 mL) was prepared by the pharmacy of the 'Assistance Publique-Hopitaux de Paris' and administered through the pumps at a mean flow rate of 2.5 μL/h. These As_2O_3-treated animals also received IFN-α (Roferon-A, Roche) subcutaneously at 10^5 UI/day per monkey for 2 weeks. Blood samples were taken during and at the end of the study. All the procedures for animal handling were performed in accordance with the regulations of the Ethics and Animal Use Committees of the Pasteur Institute.

2.5. Pharmacokinetics and laboratory analysis

The pharmacokinetics of As_2O_3 were studied in all treated monkeys. Blood samples were collected in heparinized tubes on days 0, 3, 7, 10, 14 and 45 and the residual As_2O_3 concentrations were determined by electrothermal atomic absorption spectrometry on a 5100 spectrometer with Zeeman effect background correction system (Perkin-Elmer, Les Ulis, France), as previously described (Hermine et al., 2004). In order to evaluate the renal and hepatic toxicity of the treatment, 1 mL of serum was used to measure uremia, and the serum levels of creatinine, alanine and aspartate aminotransferases and alkaline phosphatase. From heparinized tubes on days 0, 14 and 45, blood smear were fixed with methanol and stained using Ral 555® (fast stain of May-Grünwald Giemsa). Typical "flower cells" were then counted (for a typical flower cell, see Fig. 4B). Means and SEM were calculated from six different fields. *p*-Values were obtained using the statistical Student's *t*-test.

2.6. Cell isolation and flow cytometry

PBMCs were separated from blood containing EDTA by sucrose density centrifugation on a Ficoll-Paque gradient (Sigma–Aldrich Co., St. Louis, MI) and were washed three times with phosphate-buffered saline. Lymphocyte subsets were determined by flow cytometry analysis with a FACScan (Becton Dickinson, Mountain View, CA). The cells were then washed with phosphate-buffered saline containing 7% normal goat serum (Sigma–Aldrich Co.) and incubated for 30 min at room temperature with various combinations of fluorescence-conjugated monoclonal antibodies. The stains used throughout the study were phycoerythrin-conjugated CD3 monoclonal antibody (clone SP4 from BD-pharmingen), FITC-conjugated CD4 (clone SK3 from Becton Dickinson), and polyclonal CD8 antibody produced in our laboratory. Anti-mouse IgG phycoerythrin-conjugated $F(ab')_2$ fragment (Becton Dickinson) was used as a secondary antibody to stain $CD8^+$ T-cells. Cells were run un-gated. For analysis, lymphocyte gates were set with CellQuest™ software (Becton Dickinson). Lymphocyte subpopulations were calculated from the total white blood cell values obtained in a complete blood count performed with PENTRA 60 C+ (ABX Diagnostics, Montpellier, France).

2.7. HTLV-1 proviral load and T-cell clonality

High molecular weight genomic DNA was extracted from PBMCs as previously described (Kazanji et al., 1997c). The HTLV-1 proviral load was measured as previously reported by an accurate, reproducible, quantitative PCR method which involves a dual-labelled fluorogenic probe (ABI PRISM 7700 Sequence detection system) (Mortreux et al., 2001). The lower limit of detection of this technique is 10 copies per 1.5×10^5 PBMCs.

The clonality of HTLV-1-infected cells was assessed by the sensitive quadruplicate elongation-mediated PCR (LMPCR) method as described elsewhere (Cavrois et al., 1996a,b, 1998; Leclercq et al., 1998, 1999).

3. Results

3.1. Effect of As_2O_3 alone or in combination with IFN-α on HTLV-1-transformed monkey cells in vitro

To investigate the effect of As_2O_3, alone or combined with IFN-α, on HTLV-1-transformed squirrel monkeys cells in vitro, we conducted a series of XTT assays, which allow measurement of cellular proliferation and viability after drug treatment. As shown in Fig. 1, the HTLV-1-transformed monkey cell line (EVO/1540) showed a significant reduction in cell prolifera-

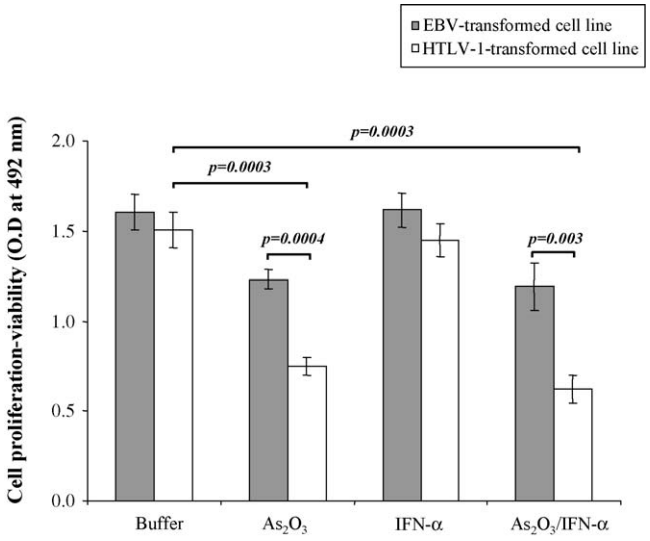

Fig. 1. Sensitivity of HTLV-1 or EBV (Epstein–Barr virus)-transformed monkey cell lines to As_2O_3 in combination with IFN-α in vitro. Cells were treated with buffer control, As_2O_3, IFN-α, or As_2O_3/IFN-α for 60 h and then processed with the XTT assay kit. Each panel represents cellular proliferation-viability by the measurement of the orange Formazan dye in a Tetrazolium salt XTT assay (optical density (O.D.) at 492 nm). The data presented here are the mean ± S.E.M. of three independent experiments. EBV-transformed monkey cell line were used as HTLV-1 uninfected control cell line. The HTLV-1-transformed monkey cell line named EVO/1540 was previously described (Kazanji et al., 1997a,b,c). Statistical analyses were performed by the Student's *t*-test, and the correspondent *p*-values are indicated.

tion and viability after treatment with As_2O_3 or As_2O_3/IFN-α ($p = 0.0003$ for both). In contrast, incubation of the cells in culture medium or with IFN-α alone had no effect. Monkey cells transformed by Epstein–Barr virus were used as controls. Comparison of these two different transformed cells shows that reduction in cell proliferation and viability in the EBV-transformed cells also occurs, but is much lower than in EVO/1540 cells after As_2O_3 ($p = 0.0004$) or As_2O_3/IFN-α ($p = 0.003$) treatment.

3.2. Pharmacokinetics and laboratory results in As_2O_3-treated monkeys

We then tested the effect of As_2O_3 or As_2O_3/IFN-α in vivo. In a first series of experiments, two monkeys (94143 and 94083) were treated for 1 month with 0.9 and 0.3 mg/day of As_2O_3, respectively. As shown in Fig. 2A, high levels of As_2O_3 were detected in the blood of these two animals, which correlated with the doses received. The monkey treated with 0.9 mg/day died 3 weeks after treatment. The hematological parameters observed in this animal at 2 weeks after initiation of the treatment showed a severe anemia associated with leucopenia and thrombocytopenia. Biochemical analyses revealed a kidney failure associated with hepatic dysfunctions (data not shown) and histological observations at necropsy showed that death of this monkey was the result of the arsenic toxicity. The second monkey survived but showed similar changes in hematological and biochemical parameters (data not shown) and was sacrificed 28 days after the beginning of treatment for ethical reasons.

In the second set of experiments, six squirrel monkeys were used. Four (1491, 1540, 1715, 94119) were infected with HTLV-1, and two (A025C and 94041) were used as controls. All six monkeys received As_2O_3 at a dose of 0.14 mg/day for 2 weeks. Pharmacokinetic analysis showed that As_2O_3 was present in all treated animals 3 days after treatment, with the highest blood level (0.4–0.8 μmol/L) on day 7. The As_2O_3 blood levels had decreased by day 10 and became undetectable by day 45 (<0.1 μmol/L) after treatment. Treated monkeys did not show any clinical or laboratory toxicity.

3.3. Changes in T-cell ($CD3^+$, $CD4^+$ and $CD8^+$) counts in HTLV-1-treated monkeys

Lymphocyte subpopulations ($CD3^+$, $CD4^+$ and $CD8^+$) were evaluated 0, 3, 7, 10, 14 and 45 days after the beginning of treatment. As seen in Fig. 3, the absolute number of total T

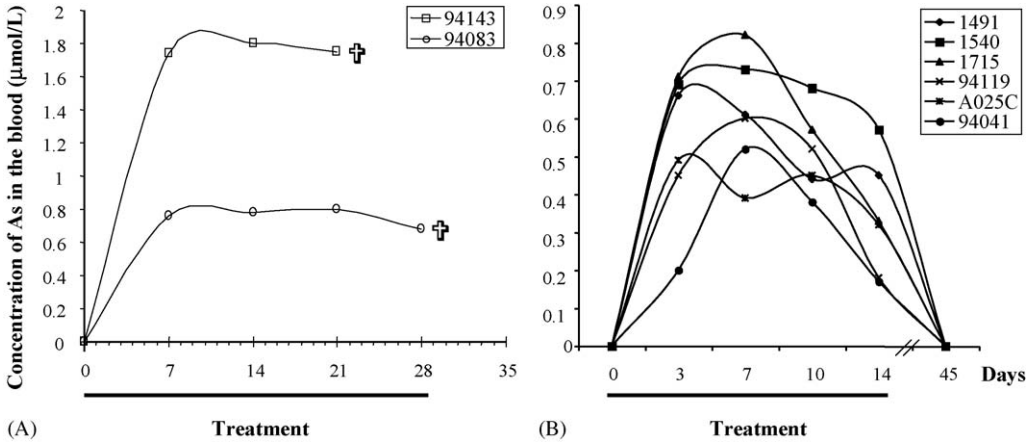

Fig. 2. Individual kinetics of blood arsenic in μmol/L during As_2O_3 or As_2O_3/IFN-α treatment. (A) Two HTLV-1 uninfected monkeys #94143 and 94083 were treated with As_2O_3 at 0.9 and 0.3 mg/day, respectively, for 1 month. Animal 94143, which received the highest dose, died 21 days after the treatment, and animal 94083 was sacrificed 28 days after the treatment. (B) Blood arsenic concentrations during the 14 days of treatment and on day 45 after treatment. Four animals (1491, 1715, 1540 and 94119) were chronically infected with an HTLV-1-transformed simian cell line, and two HTLV-1 uninfected monkeys (A025C and 94041) were used as negative controls. All monkeys were treated for 14 days with 0.14 mg/day of As_2O_3 (total of 2 mg per monkey over 2 weeks).

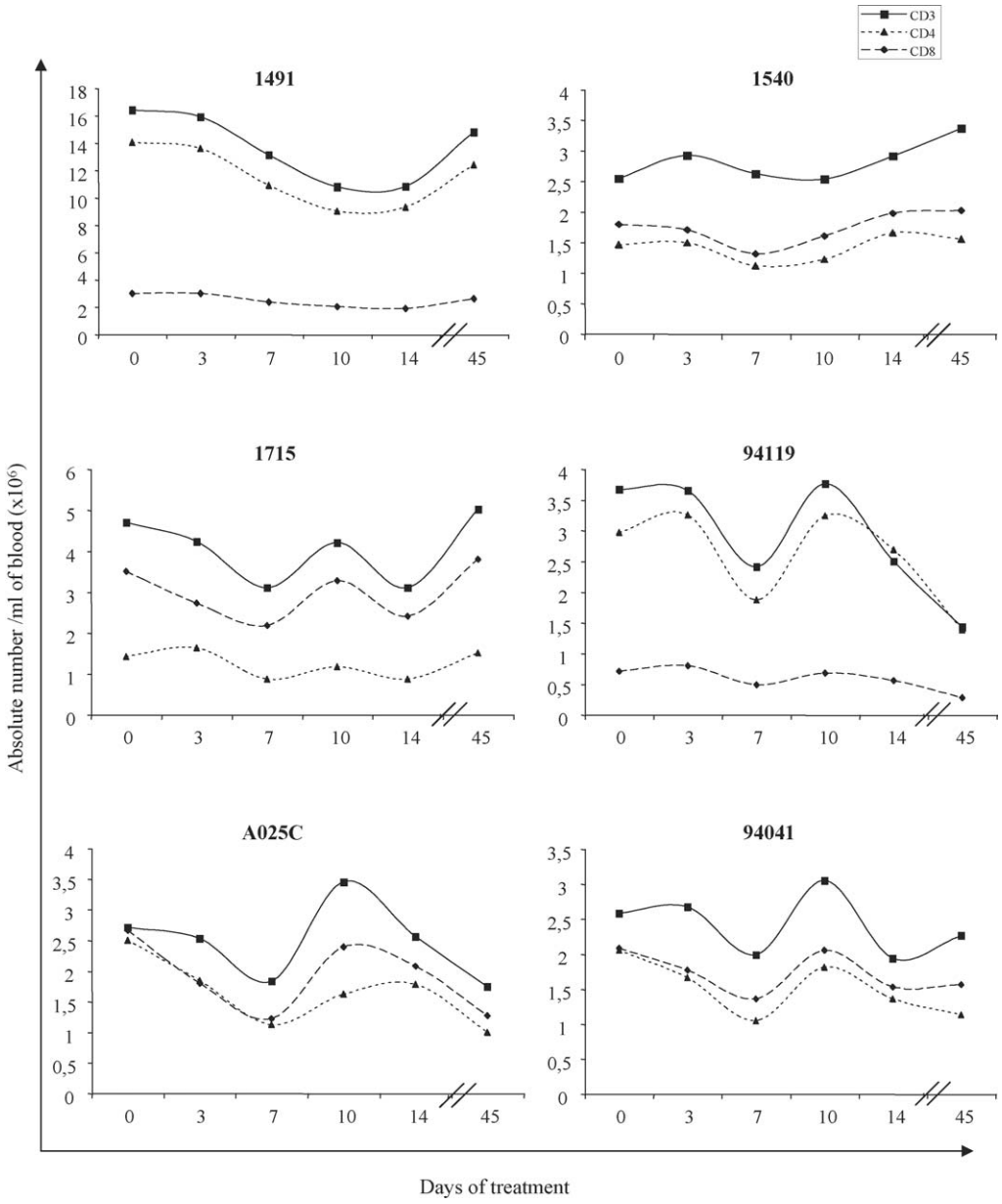

Fig. 3. Absolute numbers of peripheral blood $CD3^+$, $CD4^+$ and $CD8^+$ cells during As_2O_3/IFN-α treatment in squirrel monkeys. Four animals (1491, 1715, 1540 and 94119) were chronically infected with an HTLV-1-transformed cell line, and two uninfected monkeys (A025C and 94041) were used as negative controls. $CD3^+$, $CD4^+$ and $CD8^+$ cells were counted at days 0, 3, 7, 10 and 14 during treatment and at 45 days after treatment, with a cell counter and by flow cytometry.

lymphocytes ($CD3^+$) had declined rapidly by day 3 after treatment began, both in HTLV-1-infected and HTLV-1 uninfected animals. The lowest absolute number was seen 7 days after the beginning of treatment. The absolute lymphocyte numbers returned to normal 45 days after treatment, except in one infected (94119) and one control animal (A025C), in which the absolute number remained low. When we evaluated the changes in $CD4^+$ and $CD8^+$ cells counts during the same period, all animals showed decreased numbers of both cell populations between days 0 and 7 (Fig. 3). By day 45 after the initiation of the treatment, the two animals with low total T lymphocyte counts (94119 and A025C) also showed persistently low $CD4^+$ and $CD8^+$ counts, whereas the other monkeys had normal levels.

3.4. HTLV-1 proviral load, clonality and the presence of flower cells in infected monkeys

To evaluate the direct effect of As_2O_3/IFN-α treatment, the HTLV-1 proviral load was evaluated in the four infected monkeys. As shown in Table 1, the treatment had no significant effect on the percentage of HTLV-1-infected PBMCs evaluated at 0, 14 and 45 days after treatment. However, in animal 1491 we noted a constant lymphocytosis ($>10 \times 10^9$/L with a $CD4^+$ phenotype) that was associated with high proviral load. The elevated proviral load in this animal was also correlated with the persistent proliferation of a restricted number of circulating T-cell clones as demonstrated by LMPCR analysis of HTLV-1 integration sites in PBMCs (Fig. 4A). These findings mirror previous observations

Table 1
HTLV-1 proviral load before, during and after the treatment with As_2O_3 and IFN-α in squirrel monkeys

Monkey #	% of Proviral load (days after treatment)		
	0	14	45
1491	6.6 ± 0.9	7.3 ± 0.2	7.5 ± 0.9
1540	0.9 ± 0.1	0.8 ± 0.08	0.7 ± 0.09
1715	4.2 ± 1.2	4.0 ± 0.2	5.9 ± 0.6
94119	0.15 ± 0.03	ND	ND

Table 2
Evolution of absolute numbers of flower cells in monkey 1491 at days 0, 14, and 45 of As_2O_3 and IFN-α treatment

Days after treatment	Absolute number of "flower cells" ($\times 10^6$/mL of blood)	Decrease in absolute number of "flower cells" (%)
0	1.19 ± 0.33	0
14	0.46 ± 0.10	61 ($p = 0.0004$)
45	0.84 ± 0.24	29 ($p = 0.058$)

For the "flower cells" values, data presented here are the mean ± S.E.M. for six different countings. Statistical analysis was performed by a Student's *t*-test.

that were made during human HTLV-1 infection (Cavrois et al., 1996b; Leclercq et al., 1998, 1999) and suggests a selection of specific T-cells in vivo that is similar to some pre-ATLL conditions (Chen et al., 1995). Furthermore, we also found abnormal lymphocytes with convoluted nuclei (flower-like cells) in this animal (Fig. 4B). The number of flower-like cells had declined significantly (by 61%) ($p = 0.0004$) by day 14 after As_2O_3/IFN-α treatment and returned to the pretreatment level following suspension of the drugs (Table 2).

4. Discussion

In this study, we have shown that treatment with As_2O_3 in combination with IFN-α induces growth arrest and apoptosis in a

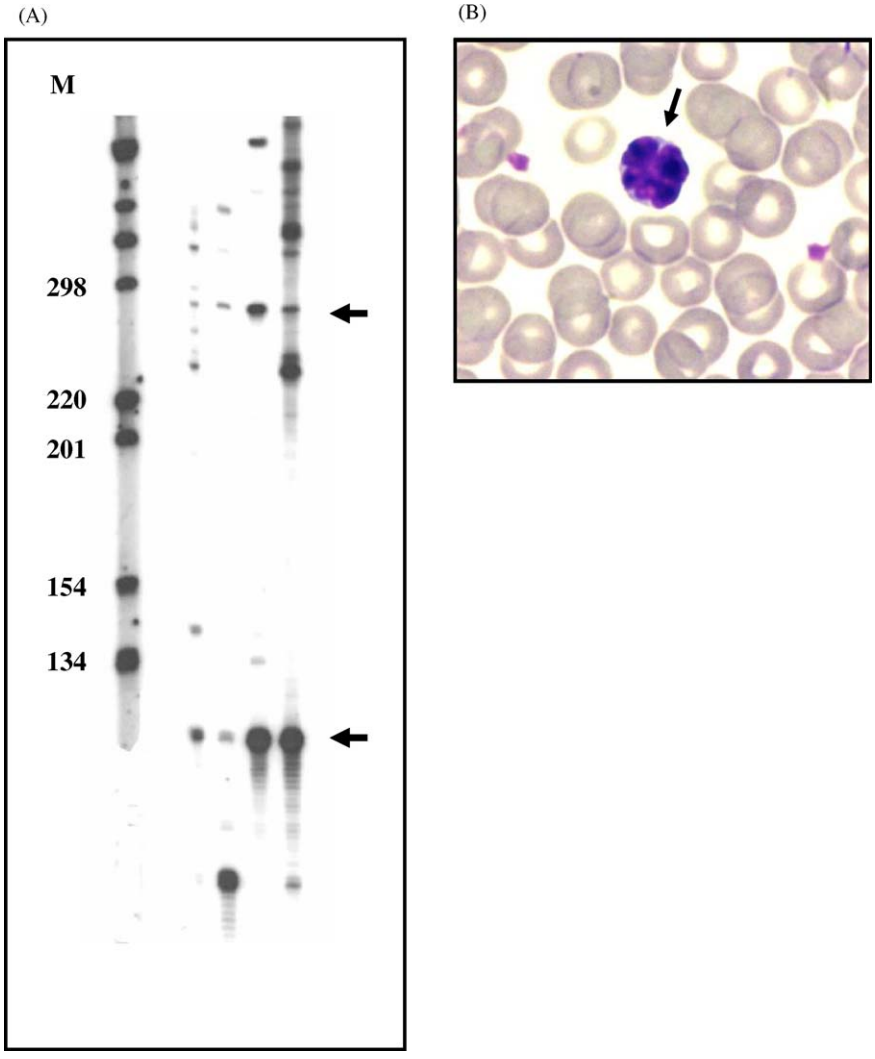

Fig. 4. Molecular and cellular pattern of a monkey affected by an ATL-like disease. (A) Quadruplicate LMPCR analysis of HTLV-1 integration sites in DNA sample from 1491 experimentally infected squirrel monkey. M, molecular weight marker (positions are indicated in base pairs). (B) A flower cell from the same 1491 HTLV-1-infected monkey (arrow) as visualized under the light microscope. Blood smears were fixed with methanol and stained using Ral 555® (fast stain of May-Grünwald Giemsa).

monkey cell line transformed by HTLV-1 in vitro. These results confirm previous reports on the efficacy of As_2O_3/IFN-α on human HTLV-1-transformed cells and on fresh ATLL leukemia cells in vitro. Thus, as in humans, As_2O_3 in combination with IFN-α killed HTLV-1-transformed simian cells, possibly via the Tax down-regulation and the reversal of NF-κB activation (Bazarbachi et al., 1999; El-Sabban et al., 2000; Li and Gaynor, 1999; Mahieux et al., 2001; Nasr et al., 2003).

We also showed a beneficial effect of As_2O_3/IFN-α treatment in vivo in a non-human primate model of HTLV-1 infection. Although no significant reduction in the proviral load was observed, As_2O_3/IFN-α treatment significantly reduced the total number of circulating HTLV-1 flower cells. This was associated with a significant reduction in the absolute numbers of $CD4^+$ and $CD8^+$ cells. We showed previously in this model that HTLV-1-infected animals develop an anti-Tax and anti-Env cytotoxic response that can be detected 2 months after infection and remain thereafter (Kazanji et al., 2000). A similar CTL response was also detected in TSP/HAM patients as well as in asymptomatic HTLV-1 carriers. Several authors have suggested that this anti-Tax CTL plays a major role in controlling HTLV-1 replication (Bangham, 2000). We previously demonstrated that PBMCs, spleen and lymph nodes are major reservoirs for HTLV-1 during the early phase of infection in our squirrel monkey model of HTLV-1 infection (Kazanji et al., 2000). Hence, elimination of $CD8^+$ cells by As_2O_3/IFN-α treatment in squirrel monkeys could stimulate the viral replication in the various lymphoid organs that represent the major reservoir of the virus. This might increase viral production and therefore result in expansion of the number of newly infected cells. Given the fact that AZT prevents cells from being infected with HTLV-1 in vitro, treatment based on a combination of As_2O_3 and AZT might be expected to induce cell death in HTLV-1-infected cells and prevent new infection.

Interestingly, in our study, a significant reduction in HTLV-1 flower cells was observed in one animal in which permanent lymphocytosis and circulating polyclonal HTLV-1-infected clones had been previously reported (Mortreux et al., 2001). Bazarbachi et al. (1999) have reported that the As_2O_3/IFN-α combination specifically induces the degradation of the viral oncoprotein Tax by the proteasome, resulting in reversal of NF-κB activation. Proteasome-mediated degradation of Tax by arsenic/IFN is reminiscent of the proteasome-mediated degradation of PML-RAR by arsenic in acute promyelocytic leukemia. Nevertheless, expression of Tax in ATLL patients is controversial. It has been reported that Tax-specific CTLs are responsible for continuous clearance of Tax-expressing cells in vivo (Hanon et al., 2000), and the existence of Tax-specific CTLs has also been demonstrated in ATLL patients, strongly suggesting Tax expression in at least some ATLL cells (Arnulf et al., 2004). Thus, degradation of the Tax oncogene might be the basis of the therapeutic response, as is also attested by reversal of NF-κB activation by As_2O_3/IFN-α in fresh ATLL leukemia cells.

Future studies should evaluate the use of intermittent administration of arsenic (to diminish toxicity) and the best timing for arsenic therapy: frontline with AZT or IFN-α or later as maintenance therapy in combination with AZT or IFN-α. These results strengthen the concept of oncogene-targeted cancer therapy.

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