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Affiliations

Katsuji Shinagawa, Okayama University Hospital, Okayama; Masamitsu Yanada and Nobuhiko Emi, Fujita Health University School of Medicine, Toyoake; Toru Sakura, Saiseikai Maebashi Hospital, Maebashi; Yasunori Ueda, Kurashiki Central Hospital, Kurashiki; Masashi Sawa, Anjo Kosei Hospital, Anjo; Junichi Miyatake, Kinki University Faculty of Medicine, Osakasayama; Nobuaki Dobashi, Jikei University School of Medicine; Yoshihiro Hatta, Nihon University School of Medicine; Yukio Kobayashi, National Cancer Center Hospital, Tokyo; Minoru Kojima, Tokai University School of Medicine, Isehara; Shigehisa Tamaki, Ise Red Cross Hospital, Ise; Hiroshi Gomyo, Hyogo Cancer Center, Akashi; Etsuko Yamazaki, Graduate School of Medicine and Faculty of Medicine, Yokohama City University, Yokohama; Katsumichi Fujimaki, Fujisawa City Hospital, Fujisawa; Norio Asou, International Medical Center, Saitama Medical University, Hidaka; Keitaro Matsuo, Kyushu University Faculty of Medical Sciences, Fukuoka; Shigeki Ohtake, Kanazawa University Graduate School of Medical Sciences, Kanazawa; Yasushi Miyazaki, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki; Kazunori Ohnishi, Hamamatsu University School of Medicine, Hamamatsu; and Tomoki Naoe, Nagoya University Graduate School of Medicine and National Hospital Organization Nagoya Medical Center, Nagoya, Japan.

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MYELODYSPLASTIC SYNDROMES (M SEKERES, SECTION EDITOR)

Lessons from the Atomic Bomb About Secondary MDS

Tomoko Hata · Daisuke Imanishi · Yasushi Miyazaki

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Abstract Myelodysplastic syndromes (MDSs) is a hematological neoplasm defined by ineffective hematopoiesis, dysplasia of hematopoietic cells, and risk of progression to acute leukemia. MDS occurs as de novo or secondary, and chemoradiotherapy for cancers is thought to increase the risk of MDS among patients. Recently, an epidemiological study for MDS among A-bomb survivors was performed, and it clearly demonstrated that the exposure to external radiation significantly increased the risk of MDS. Precise epidemiological data among survivors have revealed important clinical factors related to the risk of leukemias. In this review, by comparing data for secondary MDS and leukemia/MDS among survivors, several factors which would affect the risk of MDS, especially secondary MDS, are discussed.

Keywords Myelodysplastic syndromes · Hematopoiesis · Hematopoietic cells · Atomic bomb survivors

Study for Hematological Malignancies Among A-Bomb Survivors

In 1945, A-bomb exploded at Hiroshima and Nagasaki in Japan. To investigate lifelong health effects of A-bomb (mostly effects of radiation), a study program called the Life Span Study (LSS) was established in 1950, 5 years after bombing. In LSS, a cohort containing about 94,000 survivors (in Hiroshima and Nagasaki) and 27,000 control individuals (not in cities at the time of bombing) has been followed since then. Detailed interviews from the participants about the situations at bombing allowed radiation dose estimation. A lot of data regarding long-term effects of radiation on human were shown using epidemiological data from the LSS cohort [1]. The follow-up of this cohort is still ongoing.

In the field of hematology, the most striking data from LSS were the increased risk of leukemia among survivors. It was published as a part of LSS project in "Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950–1987" [2]. This study clearly demonstrated that ionizing radiation increases the risk of three types of leukemia: acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML). Dose response and the effects of several factors on the risk of leukemia were different among the three subtypes, and the trend of the risk along with attenuated age differed among ALL, CML, and AML. Follow-up data of leukemia risks were recently published [3••] and showed that the leukemia risks, especially for AML, still remain to be significantly increased among survivors even 55 years after the bombings.

The myelodysplastic syndromes (MDSs) were originally systematically classified by the French-American-British (FAB) group in 1982 [4], which categorized them as myeloid neoplasms typified by dysplasia, ineffective hematopoiesis, and pre-leukemia. Although both refractory cytopenia to ordinary treatment and pre-leukemic hematological conditions were recognized among hematologists at that time, these two features were combined into subtypes of MDS by the FAB group. Before the FAB classification for MDS was published, it was difficult to describe *MDS* as a defined category of hematological disorders. The original LSS study for hematological malignancies lacked MDS as a target disease.

Chemoradiotherapy and MDS

Recent studies have deepened our understanding of the important roles of genetic and epigenetic abnormalities in MDS [5]. Mutated genes coding epigenetic regulators, splicing machineries, transcription factors, and others were reported.

T. Hata · D. Imanishi · Y. Miyazaki (✉)
Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University, 1-12-4 Sakamoto, Nagasaki, Nagasaki 852-8523, Japan
e-mail: y-miyaza@nagasaki-u.ac.jp

Well-known causes of MDS for adults are environmental factors such as history of chemoradiotherapy, radiation, and tobacco smoking. While anticancer agents and ionizing radiation damage DNA of malignant cells to induce clinical effects therapeutically, it is assumed that they also induce genetic damage in normal hematopoietic cells, resulting in the increased incidence of secondary hematological disorders including MDS. For example, chemotherapy and radiation therapy for non-Hodgkin's lymphoma or breast cancer were shown to increase the risk of MDS (and leukemia) [6, 7]. However, the clearest evidence for the risk of MDS by radiation has come from A-bomb survivor data.

MDS Among A-Bomb Survivors

There has been a concern regarding MDS risk among survivors for a long time [8]. However, this had not been rigorously studied, partly because MDS was not included in LSS cohort data as a clinical entity until recently. When the LSS started in 1950, the disease concept of MDS was not established yet, and clinical features of MDS were not as clearly understood as leukemia among hematologists before the mid-1980s, resulting in difficulty in diagnosing MDS. To study the relationship between radiation and the risk of MDS among survivors, Atomic Bomb Disease Institute (ABDI) data set in Nagasaki University and LSS-Nagasaki data were used to identify MDS among survivors [9••]. The ABDI database contains routine health data for survivors. Although the ABDI data set lacked individual radiation dose estimation, the ABDI cohort is larger than the LSS-Nagasaki cohort. For the analysis of ABDI data, the distance from the A-bomb impact hypocenter, instead of individual radiation dose, was used to estimate the radiation exposure. Both distance from the hypocenter and the individual dose were used for the analysis of LSS-Nagasaki data.

There were 151 cases of MDS among 64,026 survivors in the ABDI cohort and 47 MDS cases in 22,245 survivors in the LSS-Nagasaki cohort (Table 1). The median age at diagnosis was 71 and 72.4 years for ABDI MDS and LSS-Nagasaki MDS, respectively. By using two data sets independently, statistically significant relationships were found between the excess relative risk (ERR) of MDS and radiation dose and distance from the hypocenter (an inverse relationship, in this case). It was shown that higher-risk MDS subtypes, such as refractory anemia (RA) with excess blasts (RAEB) and RAEB in transformation (RAEB-t), occurred more commonly than RA or RA with ringed sideroblasts. There was also an age effect; those exposed younger than 20 years old had a greater risk of developing MDS. The ERR was 4.3 per Gy, with a linear dose-response pattern.

Several Considerations for MDS Among Survivors Compared with Therapy-Related, Secondary MDS

There are many reports describing secondary myeloid malignancies after chemotherapy and/or radiation therapy for a primary malignancy. Insight can be gained into the pathology of these diseases by comparing several MDS factors among A-bomb survivors and those developing MDS after chemotherapy or radiation therapy for another cancer, in elucidating what we know about *secondary* MDS.

Age at Diagnosis of MDS

The median age at diagnosis of de novo MDS is approximately 70 years; among the more than 7000 de novo MDS patients worldwide included in the revised international prognostic scoring system (IPSS-R), it was 71 years [10]. The median age of *therapy-related* MDS cases differs based on the primary disease and therapeutic modality. For example, in a study of therapy (radiation alone and combined with chemotherapy)-related MDS and AML among breast cancer patients, the median age of MDS/AML was 57, the same as that of the total primary cohort, reflecting the biology of breast cancer itself [7]. In a report of MDS after autologous transplantation for lymphoma, the median age at diagnosis of MDS was 44 years old [11]. This was younger than that of a de novo MDS cohort and of the abovementioned report, probably because autotransplantation was administered to young, but not elderly, patients. In one report, comparing MDS risk of prostate cancer patients treated with radiation alone or hormone therapy and radiation to MDS risk among patients treated with surgical resection alone, there was no statistically significant increase of MDS risk among patients treated with radiation therapy. The median age of these study subjects was 64 years [12]. Among A-bomb survivors, MDS patients were as old as de novo MDS. This is despite the fact that the age of survivors varied widely, reflecting the almost natural distribution in age groups seen in Japan at that time.

Age is an important factor when discussing the risk of radiation-induced hematological malignancies. As previously shown in studies from the LSS cohort, age at exposure had significant effects on the risk of ALL and AML [2, 3]. In ALL, younger people had the greater risk. However, in AML, though the different age groups had different levels of risk, a simple age-risk relationship did not exist. For CML, there has been no age effect on the risk. These data demonstrated that age at the time of exposure to radiation contributed to the risk of leukemia differently by type of leukemia.

Among survivors, younger people had a greater ERR for MDS, though mathematical modeling for age group was not done [9••], which made it difficult to compare the age effect on the risk of MDS with those of leukemias. Although not much attention has been paid to the age of patients, it might have

Table 1 Hematological malignancies among A-bomb survivors

	ABDI cohort				LSS-Nagasaki cohort			
	Exposure distance (km)				Weight bone marrow dose (Gy)			
	<1.5	1.5–2.99	≥3	Total	≥1	0.005–0.999	<0.005	Total
Male								
Population at risk	1693	6485	16,092	24,270	273	2665	5904	8842
No. of patients	12	21	34	67	3	8	10	21
Person-years	23,071	91,880	233,191	348,144	2959	29,789	66,102	98,850
Crude rate	52	22.9	14.6	19.2	101.4	26.9	15.1	21.2
Female								
Population at risk	2258	10,663	26,835	39,756	351	4201	8851	13,403
No. of patients	13	23	48	84	5	7	14	26
Person-years	34,946	158,144	405,980	599,071	4480	52,926	114,363	171,769
Crude rate	37.2	14.5	11.8	14	111.6	13.2	12.2	15.1

greater effects than we expect on the risk of therapy-related hematological disorders and even on the types of disease.

Gender of Patients

In malignancies among survivors, it is known that gender has a significant impact on risks of developing solid cancers and leukemias. ERR of all solid cancers for females was 0.58/Gy, which is higher than that for males (0.35/Gy). This pattern does not change, even when excluding gender-specific cancers (breast, ovary, and testis); the ERR was 0.61 and 0.34/Gy for females and males, respectively [13]. However, among leukemias, male gender showed higher risks for ALL and CML, but there was no difference by gender for AML [2, 3]. In the IPSS-R publication, male/female ratio of all types of de novo MDS was 1.56 [10]. For MDS among survivors, the crude incidence rate was higher for males than for females in both ABDI and LSS-Nagasaki cohorts (19.2 and 14.0 among 100,000 person-years in ABDI and 21.2 and 15.1 in LSS-Nagasaki, respectively); however, statistical significance in the crude relative risk (RR) was achieved only in the ABDI cohort (1.3, 95 % confidence interval, 1.0–1.9). This might be because of a smaller number of survivors in the LSS-Nagasaki than in the ABDI data.

When we discuss MDS after chemotherapy or radiation therapy, little attention has been paid to the effect of gender on the risk of developing secondary MDS. This is partly because the number of male patients (or female patients) with secondary MDS strongly relates to the gender ratio in incidence and prognostic influence of gender on the primary diseases. Although we do not know how gender affects the risk of developing MDS after chemotherapy or radiation therapy, its importance is suggested by data from hematological malignancies and solid cancers among survivors.

Time from the Events (Radiation, or Chemotherapy and Radiation) and the Delivery of Radiation

The median follow-up periods vary widely in reports of therapy-related MDS and MDS/AML. Some were around 10 years, but some less than 5 years. The median observation time was 18.5 and 16.5 years for ABDI and LSS-Nagasaki cohort, respectively, longer than most published papers on secondary MDS. It took a median of 12 years (ABDI) and 14.5 years (LSS-Nagasaki) to develop MDS since 1985, when observation started for these two cohorts, emphasizing the point that long durations of follow-up are necessary to capture secondary MDS events. It may take longer for the onset of secondary MDS caused by radiation alone than by a combination of chemotherapy and radiotherapy; however, it is a complex issue affected by the types of chemotherapy agents, the schedule and dose of chemotherapy and radiation, the path of radiation exposure, age of patients, gender, etc. How long do we need to observe patients after chemotherapy or radiation therapy to evaluate the risk of therapy-related MDS? It takes longer to develop secondary AML after administration of alkylating agents than topoisomerase inhibitors. How about radiation alone? How about for MDS? We do not know the answer, yet.

In the paper studying MDS risk among prostate cancer patients treated with external radiation alone [12], the median observation time for all subjects was 3 years, including those treated by surgical operation alone and those with interstitial brachytherapy. Although the external beam radiotherapy group had a longer observation period (8.2 years), it was still shorter than the median time to develop MDS (9.1 years). With a longer observation period, a statistically significant increase of MDS may have been realized, a point of discussion in the paper [12]. A similar study exploring the risk of AML showed a statistically significant increase of AML

following external beam radiation therapy for prostate cancer even with less median follow-up of this group [14•]. The distinct biology of AML and MDS, such as differences in time to develop these diseases, might have contributed to these results. There is no large series of data regarding MDS risk among A-bomb survivors until 1985, which means we know nothing about the incidence of MDS in the first 40 years after bombing [9••].

For MDS patients among A-bomb survivors, the exposure was external, whole body, one time, and high-dose radiation ranging from several milligrays to several grays (probably less than 5 Gy). With radiation therapy for malignancies, it is local, fractionated, relatively low-dose-rate radiation up to about 50 Gy, depending on the primary disease. We may also need to consider these factors to precisely analyze the risk of secondary hematological disorders including MDS after exposure to ionizing radiation.

For Understanding of MDS

There are many issues to be solved in understanding the biology of secondary MDS, which is necessary for proper diagnosis and management of patients and for establishing standard treatments for these patients. This will also contribute to our understanding of de novo MDS and to developing new treatments for MDS.

The type of treatment for the primary cancer affects the clinical features of secondary MDS, especially the karyotype of bone marrow cells [15, 16]. Types of prior treatments, such as alkylating agents, topoisomerase inhibitors, or radiation, correlate with cytogenetic abnormalities of secondary MDS. Although there is no specific karyotypic abnormality in secondary MDS (and secondary AML), cases with unfavorable cytogenetics (abnormalities on chromosome 7, complex karyotype, etc.) are common in this category, suggesting poor prognosis of secondary cases [16]. One recent report showed that AML and MDS after radiation therapy alone are similar to de novo diseases in terms of clinical behavior and cytogenetic abnormality, suggesting biologically different effects of radiation (for treatment of primary malignancies) on normal hematopoietic cells from chemotherapeutic agents with or without radiation [17]. MDS after radiation alone had a similar spectrum of cytogenetic abnormalities to de novo cases, and those after chemotherapy (+/−radiation) had more unfavorable karyotypes, indicating that the secondary nature of the disease had no significant impact on prognosis. In de novo MDS, cytogenetics has the greatest impact on survival [10], and prior treatment might just affect the type of genetic abnormality in hematopoietic cells in developing MDS, emphasizing the importance of genetic abnormalities themselves in defining biological and clinical characteristics of MDS. The importance of somatic mutations in MDS has also been demonstrated in the setting of allogeneic hematopoietic stem cell transplantation (allo-SCT) [18]. After day 100 of transplantation, one multivariable model identified mutations in *TP53*, *TET2*, and *DNMT3A* and complex karyotype as significant risk factors for overall survival, supporting the importance of genetic factors for survival even in the presence of expected graft versus MDS effect.

How about in MDS among survivors? So far, there are no data published regarding the clinical behavior of MDS after A-bomb exposure, including hematological parameters, analysis of karyotype in patients, transformation to leukemia, and overall survival. Evaluation of these data will be informative to demonstrate the clinical features of MDS among survivors and MDS related to radiation exposure. As mentioned above, the importance of genetic changes has become apparent, and it is necessary to explore genetic abnormalities of MDS among survivors. Clinically, this will also be important to determine whether efficacy of treatments, including azacitidine, lenalidomide, and transplantation, differs.

Finally, regarding latency of MDS among survivors, how does the higher risk of MDS (and also AML) persist for more than 50 years? Is this also true for those who received chemotherapy and radiation therapy? This may relate to a fundamental question of how MDSs (and most hematological neoplasms) develop in the elderly.

MDS among A-bomb survivors is unique in terms of its clear etiology based on a solid epidemiological analysis. Making its clinical and biological characteristics clear, including response to treatments, would provide important and useful information to consider other secondary MDS and therapy-related myeloid neoplasms.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Tomoko Hata, Dr. Daisuke Imanishi, and Dr. Yasushi Miyazaki each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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LETTER TO THE EDITOR

Molecular analysis of loss of CCR4 expression during mogamulizumab monotherapy in an adult T cell leukemia/lymphoma patient

Masataka Taguchi · Yoshitaka Imaizumi · Daisuke Sasaki · Tomonori Higuchi · Kazuto Tsuruda · Hiroo Hasegawa · Jun Taguchi · Yasushi Sawayama · Daisuke Imanishi · Tomoko Hata · Katsunori Yanagihara · Osamu Yoshie · Yasushi Miyazaki

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Dear Editor,

A 63-year-old male was admitted to our hospital with relapsed CC chemokine receptor 4 (CCR4)-positive adult T cell leukemia/lymphoma (ATL) (Fig. 1a). He received intravenous infusions of mogamulizumab (Moga), a defucosylated, humanized anti-CCR4 monoclonal antibody [1], once a week at a dose of 1.0 mg/kg. After the third infusion of Moga, morphologically abnormal lymphocyte count and human T cell leukemia virus type 1 (HTLV-1) proviral load [2] decreased from 4.8×10^9/L (32 % of white blood cell (WBC)) and 60.3 copies/100 peripheral blood mononuclear cells (PBMCs) to 0.33×10^9/L (4 % of WBC) and 17.0 copies/100 PBMCs, respectively. However, serum lactate dehydrogenase and soluble interleukin-2 receptor levels rose from 475 and 4,627 U/ml to 596 and 56,092 U/ml, respectively. His mediastinal and intra-abdominal lymph nodes also increased in size. Flow cytometric analysis (FCM) of his PB revealed that the majority of the remaining ATL cells were negative for CCR4 (Fig. 1a). It should be noted that the anti-CCR4 antibody used for FCM was clone lG1, which binds to a different epitope from Moga [3]; thus, epitope masking by Moga was unlikely. Southern blot hybridization analysis showed the same monoclonal integration of the HTLV-1 provirus as before (Fig. 1b), which indicated that ATL cells from the pre- and post-Moga monotherapies were of the same clonal origin.

To elucidate the molecular mechanisms underlying the loss of CCR4 antigen expression, we analyzed the messenger RNA (mRNA) expression of *CCR4* and other related genes in his PBMCs using reverse transcriptase–polymerase chain reaction analysis [4, 5] (Fig. 1c). The expression of *CCR4* mRNA markedly decreased following Moga monotherapy. On the other hand, the expression of *FRA-2*, the upstream transcription factor that induces the expression of *CCR4* and promotes cell growth in ATL [4], was maintained even after the treatment. Expression of the other target proto-oncogenes of *FRA-2*, such as *c-MYB*, *MDM2*, *BCL-6*, and *SOX4* [4, 5], was also maintained. We performed Sanger sequencing of the *CCR4* gene using genomic DNA from pre- and post-treatment PBMCs. No acquired mutations were detected in the post-treatment sample. These results suggested that neither genetic mutations nor the reduced expression of upstream transcription factors may be the cause of the loss of CCR4 expression. Epigenetic mechanisms or clonal selection may be the cause, namely a minor fraction of CCR4-negative sub-clones could have already existed and expanded during the treatment (Fig. 1a).

The loss of target molecules on tumor cells has been reported as an important mechanism of resistance to antibody-based therapies [6]. CCR4 is frequently expressed on ATL cells [7] and is a promising target molecule for therapy against ATL [1]. However, as in the case considered here, loss of CCR4 antigen expression was observed in an

M. Taguchi · J. Taguchi · T. Hata · Y. Miyazaki
Department of Hematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Atomic Bomb Disease Institute, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Y. Imaizumi (✉) · Y. Sawayama · D. Imanishi
Department of Hematology, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan
e-mail: y-imaizm@nagasaki-u.ac.jp

D. Sasaki · K. Tsuruda · H. Hasegawa · K. Yanagihara
Central Diagnostic Laboratory of Nagasaki University Hospital, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

T. Higuchi · O. Yoshie
Department of Microbiology, Faculty of Medicine, Kinki University, Osaka, Japan

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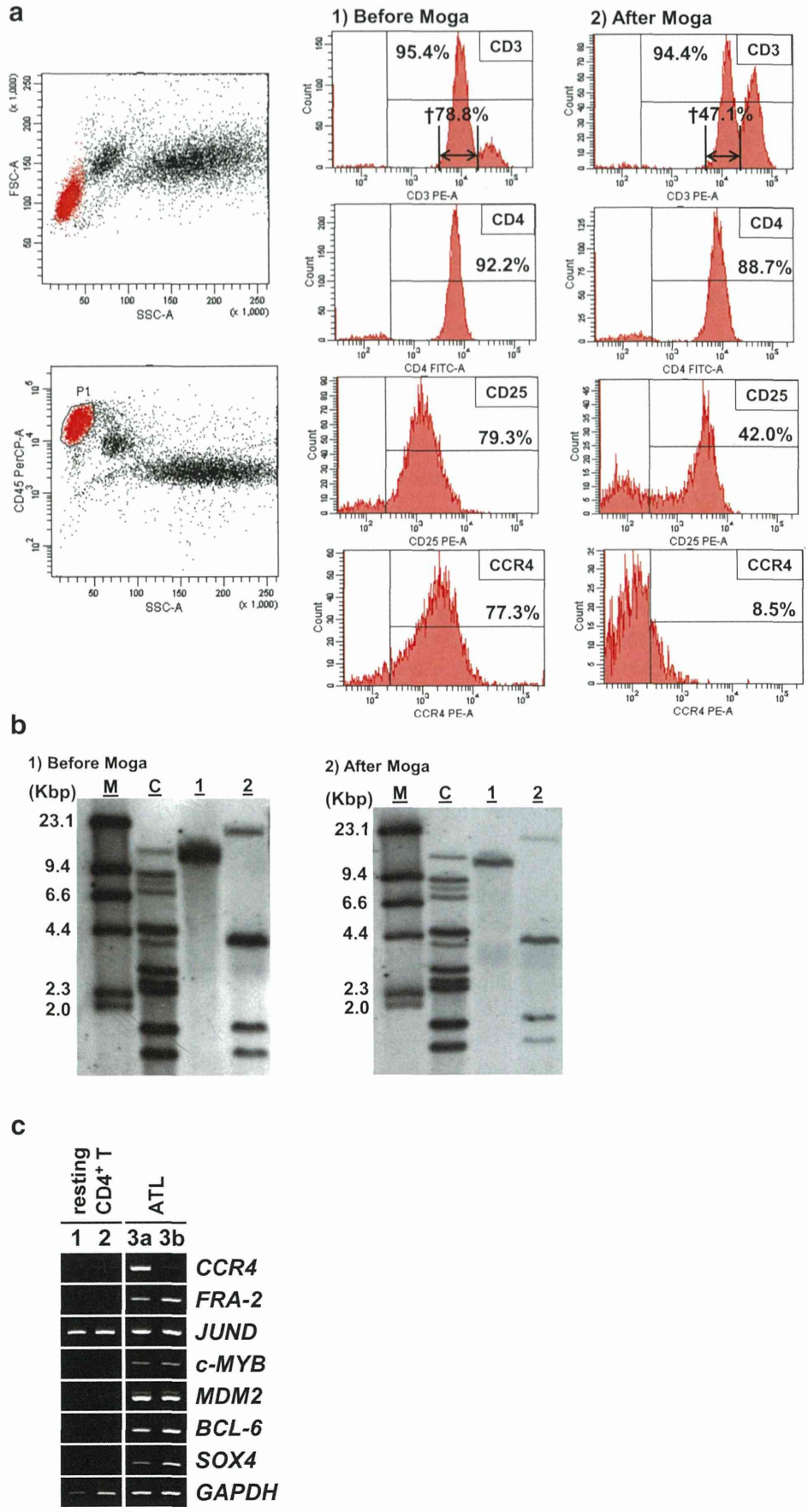

Fig. 1 **a** FCM. Lymphocytes were gated in FSC/SSC and CD45/SSC cytograms. Each histogram was conditioned on the lymphocyte gate: *1*) Before Moga monotherapy and *2*) after Moga monotherapy. Most tumor cells that accumulated in the $CD3^{dim}$ sub-population (dagger) seemed to have lost the expression of the CCR4 antigen following Moga monotherapy. **b** Southern blot hybridization analysis. The same monoclonal integration of HTLV-1 provirus was observed in PBMCs from *1*) pre- and *2*) post-Moga monotherapies. *M* size marker (λDNA/*Hind*III), *C* positive control, *lane 1* patient's DNA digested with *EcoRI*, *lane 2* patient's DNA digested with *PstI*. **c** Reverse transcriptase–polymerase chain reaction analysis. The mRNA expression of the indicated genes was examined for normal resting $CD4^+$ T cells from two healthy donors (*lanes 1* and *2*) and the patient's PBMCs before (*lane 3a*) and after (*lane 3b*) Moga monotherapy. While the expression of *CCR4* mRNA was markedly decreased following Moga monotherapy, the expression of the upstream transcription factor, *FRA-2*, as well as its downstream target proto-oncogenes, *c-MYB*, *MDM2*, *BCL-6*, and *SOX4*, remained unchanged. *GAPDH* was used as a loading control

ATL patient at relapse following Moga monotherapy [8]. Thus, the loss of CCR4 expression on ATL cells may not be a rare phenomenon and may be critically involved in resistance to Moga. Further analyses are needed to fully understand the mechanisms underlying the loss of CCR4 expression to overcome resistance to Moga.

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Conflict of interest The authors declare no competing financial interests.

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