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Effectiveness of Amplified Natural Killer (ANK) Therapy for Adult T-cell Leukemia/Lymphoma (ATL) and Future Prospects of ANK Therapy

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ABSTRACT

Amplified Natural Killer (ANK) therapy is modified to increase the safety and efficacy of the original (LAK) immunotherapy. It is a method of removing natural killer (NK) cells from the patient's own blood, culturing and amplifying the NK cells, specifically increasing their ability to attack cancer and returning them for treatment. It is generally effective against all cancers. The two cases presented here and the other treated cases show that ANK therapy is very safe and effective against ATL. Further research suggests that ANK therapy, rather than chemotherapy, is likely to be the first-line therapy for ATL. In addition, low activity of NK cells means accumulation of bacterial load. Therefore, ANK therapy with high doses of activated NK cells may be effective not only for ATL and cancer, but also for patients with chronic bacterial and viral infections.

KEYWORDS

Amplified natural killer cell therapy; Lymphokine-activated killer cell immunotherapy; Adult T-cell leukemia; HTLV-1-Associated branching bronchioalveolar disorder

INTRODUCTION

Amplified natural killer (ANK) therapy is a method of removing natural killer (NK) cells from the patient's own blood, culturing and amplifying NK cells to specifically enhance their ability to attack cancer and returning them to the patient for treatment. It is generally effective against all cancers. In 1985, Rosenberg et al. of the National Cancer Institute (NCI) performed a treatment called lymphokine-activated killer (LAK) immunotherapy [1]. A large volume of blood of about 50 L was extracted from the patient over 5 days in a week, and lymphocytes were extracted and cultured with rIL-2 for 3 days to 4 days to induce LAK cells. These cells were then transfused back to the patient. This treatment has shown some effects, but it is not common due to its high cost and strong side effects. The next instance where this therapy was performed was in Japan in the 1990s. However, the amount of blood collected was less than one-tenth of that collected by Rosenberg et al. Moreover, the expected effect was not achieved because the number of NK cells with a strong anticancer activity was low [2]. ANK immunotherapy focuses on the fact that among the various lymphocytes, NK cells have a strong anticancer effect. The amount of

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blood collected in the studies was about 5 L; however, by increasing the number and activity of NK cells before returning them to the patient, it is possible to obtain a safe and therapeutically useful effect [3].

In this study, ANK immunotherapy was performed for adult T-cell leukemia (ATL), and it was demonstrated to be very effective and safe.

The cause of ATL is human T-cell leukemia virus type 1 (HTLV-1); the cancer is a peripheral T cell tumor with a poor prognosis that develops in HTLV-1 carriers. It was first reported by Uchiyama, Takatsuki et al. in 1977 as a T cell tumor that frequently occurs in southwestern Japan, and in 1980, the RNA retrovirus HTLV-1 was identified as the causative virus[4,5]. Based on various pathological factors and the clinical course of the disease, it is divided into 4 types: acute type, lymphoma type, chronic type, and smoldering type. Acute/lymphoma type and chronic type ATL with poor prognostic factors are classified as aggressive ATL, and chronic type/smoldering type ATL without poor prognostic factors are classified as indolent ATL. Treatment methods are decided on the basis of these classifications [6]. Among them, aggressive ATL has a very poor prognosis with a median survival time of about 10 months. Indolent ATL is treated with skin-directed treatments such as corticosteroids, external retinoids, local radiation therapy, and photochemotherapy for skin lesions. Systemic treatment includes systemic administration of steroid hormones, oral retinoids, interferon gamma, and single-agent chemotherapy, but there is no evidence if these contribute to improved survival. Aggressive ATL is treated with multidrug chemotherapy immediately after the diagnosis, and allogeneic hematopoietic stem cell transplantation is performed wherever possible, considering age and general condition. However, while it has strong side effects, it is said to have a little effect on prolonging survival.

CASES

Here, we present two impressive cases out of several ATL cases that received ANK therapy.

Case 1

Case 1 was a 70-years-old woman who we believe switched from smoldering-type to acute-type ATL. Originally a patient with ringworm infection, the patient was diagnosed with smoldering ATL in 2004. In 2007, the patient had a sharp rise in soluble IL-2 receptor levels (sIL-2R) in the serum (Figure 1), an increase in systemic lymph node sizes (Figure 2A), and exacerbation of the disease due to skin tumors (Figure 2B).

The patient's blood contained abnormal cells of lymphocytes (27%). We determined that it was a conversion from the smoldering type to the acute type. Then, the patient was treated with ANK therapy with over 15 injections from November 12, 2007 to February 15, 2008 (Figure 1). This case also showed a dramatic downregulation of sIL-2R levels (Figure 2A). The patient's skin tumor also disappeared (Figure 2C), inducing complete remission. After that, the patient required supportive care for less than 5 years, and she remained in complete remission until she died of a myocardial infarction on April 22, 2013[3].

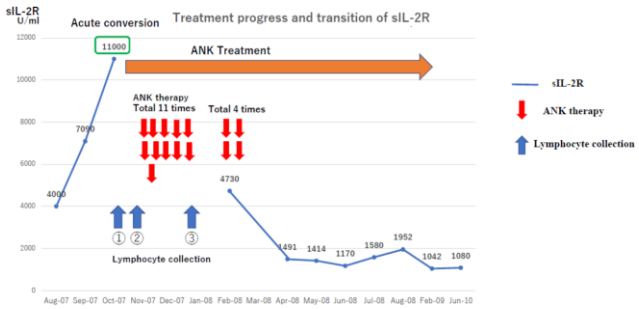

Figure 1: Soluble IL-2 receptor (sIL-2R) in the patient's serum and the course of ANK Therapy.

As sIL -2R gradually increased, ANK cell therapy was started. After repeated injections of ANK cells, sIL2R levels decreased. After the treatment, improvement of skin lesions was observed, and after that, sIL-2R remained at about 1000 to 2000.

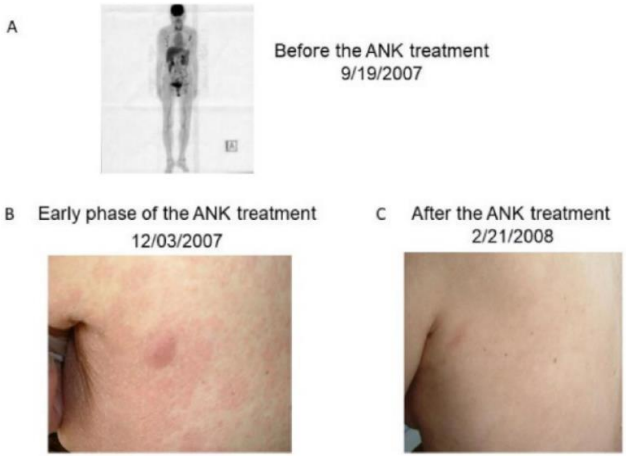

Figure 2: Positron emission tomography -computed tomography (PET-CT) images (before ANK cell therapy) and skin lesions of the patient. **A)** PET -CT showed multiple lymphadenopathy before ANK cell therapy, **B)** A rash and tumor were found throughout the body in early stages of ANK cell therapy. (December 3, 2007), **C)** After repeated administration of ANK cells, multiple skin lesions healed. (February 21, 2008).

Case 2

Case 2 was an 81-years-old woman diagnosed with HTLV-1-associated branching bronchioalveolar disorder (HABA-B) in smoldering-type ATL.

Initially, diffuse panbronchiolitis was suspected based on CT images (Figure 3) and clinical findings; however, in a respiratory function test (Table 1), the forced expiratory volume in 1s was normal, and % vital capacity (VC) was 46.5%, which was a remarkable decrease. Therefore, we suspected other diseases, and began more detailed tests. Imaging findings suggested the presence of HABA. HTLV-1 antibody tests were also positive, and the test by line blotting (almost the same test as western blotting) was positive. Therefore, the patient was judged as having active HABA. Serological examination revealed atypical lymphocytes of less than 5%, a lymphocyte count of 4000/μL or less, and lactate dehydrogenase (LDH) levels of 1.5 times or less compared to the normal levels. Serum calcium level was normal (Table 2). A bronchoscopy revealed lymphocyte infiltration from the bronchial mucosal tissue, which was consistent with HTLV-1-associated bronchioalveolar disorder (Figure 4).

Based on the above, the patient was diagnosed with a smoldering type ATL + HABA-B condition. ANK therapy (Figure 5) was performed because the patient had severe dyspnea and was elderly. There were dramatic improvements in symptoms, and in the patient's condition as observed in the chest computed tomography (CT) images (Figure 6) and respiratory function test results (Table 1) [7-9].

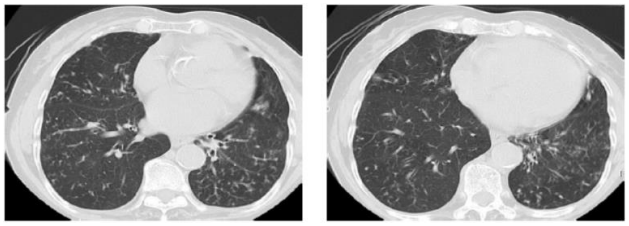

Figure 3: Chest computed tomography (CT) scan before treatment at the first visit. A) Middle lobe of lung, B) Lower lobe of lung.

Bilateral diffuse lobar central granular shadows were observed on the chest CT scan at initial visit.

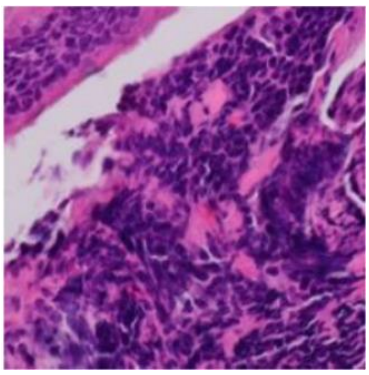

Figure 4: Bronchial mucosal findings obtained by bronchoscopy.

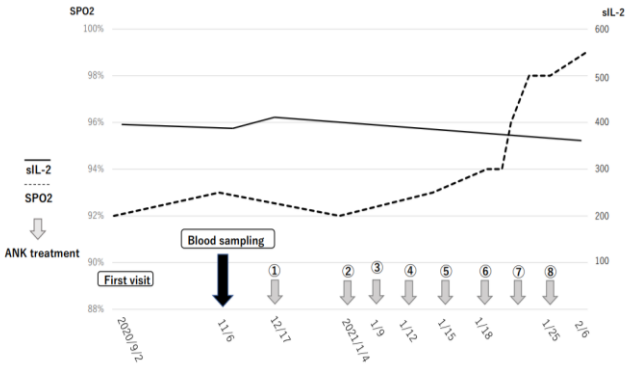

Figure 5: Treatment Course.

Numerous lymphocytic infiltrates with strong nuclear irregularity were observed in the bronchial mucosa of patient (haematoxylin and eosin stain, ×100).

Because the patient had a smoldering type human T cell leukaemia virus type 1 (HTLV) -associated bronchioloalveolar disorder, there is almost no change in sIL-2, but SpO_2 gradually improves with the course of treatment. The time from the first to the second administration is greater than other treatment intervals because an individual with suspected COVID-19 infection appeared in the patient's family preventing us from giving the amplified killer cell (ANK) treatment at the desired time (Figure 5).

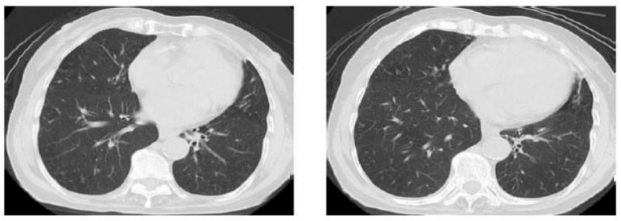

Figure 6: Chest computed tomography (CT) scan findings after treatment.

The chest CT scan showed that the bilateral diffuse lobar central granular shadows improved significantly after treatment (Figure 6).

	Before Treatment		**After Treatment**	
	Measured Value	% Predicted Value	Measured Value	% Predicted Value
VC	0.93 L	46.5	1.54 L	78.6
FVC	0.88 L	44	1.18 L	60.2
FEV1.0	0.83 L	57.6	1.12 L	82.4
FEV1.0%	94.3%	127	94.9%	128.5

Table 1: Pulmonary function tests. **Notes:** VC: Vital Capacity; FEV: Forced Vital Capacity; FEV: Forced Expiratory Volume

Hematology		**Serology**	
WBC	9540/μL	ACE	13.8/μL
		RF	4 IU/mL
Neutrophils	59%	KL-6	177 U/mL
Lymphocytes	37%	sIL-2	391 U/mL
Mono	4%	IgG	1845 mg/dL
Eos	0%	IgA	351 mg/dL
ATL	5%	IgE	2 IU/mL
Flower cells	0%	HTLV-1 Ab	(+)
RBC	3.64×10^6/μL	HTLV-1 LIA	(+)
Hb	10.6 g/dL	P.19 Ab	(+)
Plt	195 × 10/μL	P.24 Ab	(+)
Biochemistry		P.46 Ab	(+)
TP	7.3 g/dL	P.21 Ab	(+)
Alb	4.1 g/dL	T-SPOT	(-)
BUN	16.6 mg/dL	β-D glucan	2.8 pg/mL
Cre	0.62 g/dL	Cold agglu	(-)
Ca	9.3 g/dL	Intratracheal Sputum Bacteria Culture	
IP	3 g/dL	MSSA (*S. aureus*)	
T-Bil	0.7 mg/dL	Intratracheal Sputum Acid-Fast Bacilli Culture	
AST	26 U/L	(-)	
ALT	11 U/L	Non-Tuberculous Mycobacteriosis PCR test	
ALP	156 U/L	*Mycobacterium avium* (-)	
γGTP	11 U/L	*Mycobacterium intraceller* (-)	
Na	141 mmol/L		
K	3.8 mmol/L		
Cl	104 mmol/L		
CK	75 U/L		
CRP	0.28 mg/dL		
LDH	187 U/L		

Table 2: Laboratory data at initial visit.

WBC: White Blood Cells, ATL: Adult T-cell Leukemia; RBC: Red Blood Cells, Hb: Hemoglobin, Plt: Platelets, Alb: Albumin, BUN: Blood Urea Nitrogen, AST: Aspartate Transaminase, ALT: Alanine Transaminase, ALP: Alanine Phosphatase, CRP: C-Reactive Protein, LDH: Lactate Dehydrogenase; γGTP: Gamma-Glutyl-Transpeptidase.

DISCUSSION

Lymphocyte banks are involved in the production of ANK cells. ANK therapy is a treatment that takes out about 5 L of the patient's blood, amplifies the activated NK cells, and returns it to the patient. See the paper for detailed culture and production methods for ANK cells [7,8]. The reason ANK therapy was effective in these two cases is that ATL leukemia cells express NK cell co-stimulatory molecules such as CD80 and CD137L. ATL cells have the characteristics of regulatory T cells, and the immunity of ATL patients is suppressed. From this, it is clear that ATL cells express both stimulatory cofactors and suppressors such as PD-L1. It has been reported that NK cells that have been extracted from the blood and then cultured and activated can, unlike T cells, attack tumors regardless of their expression of tumor suppressor genes [10]. For this reason, ANK therapy is specific to tumor cells and has a low risk of serious damage to the normal immune system. With this treatment, patients with ATL do not experience serious side effects seen with anti-CCR4 [11,12] or anti-PD-1 therapy [13]. High expression of PD-1 in ATL cells has also been reported [14]. Therefore, anti-PD-1 antibodies may inhibit PD-1- and PD-L1-mediated negative self-regulatory signals in ATL cells and induce ATL cell proliferation. In ATL, PD-L1 may function both as an inhibitor of ATL cell proliferation and immunosuppressant for the normal immune system. It has even been reported that a large number of ANK cells kill PD-L1-positive tumor cells [15]. These findings suggest that repeated doses of NK cells, including ANK cells, alleviate immunosuppression via the PD-1-PD-L1 pathway. The onset of clinical outcomes of ATL often occurs only after a long incubation period and continues into old age. The proportion of NK cells with the CD16+CD56+ phenotype is known to be significantly lower in carriers infected with HTLV-1. Therefore, the administration of enhanced NK cells in the form of ANK therapy is considered effective [16].

The mechanism of HABA in the case 2 is described below. Even if the patient had not yet experienced the onset of ATL, the presence of ATLL pulmonary infiltrates and the opportunistic infections associated with immunosuppression created an environment in which CD8+ T lymphocytes damaged cells and induced alveolar inflammation, lymphocytic interstitial pneumonia, and various other organ disorders. The mechanism of lung lesions caused by HTLV-1 is due to the arrest of the cell cycle of T cells in the G1 phase by the Tax protein, the anti-apoptotic effect by the activation of NF-κB, and the inhibition of DNA damage repair by the suppression of p53. Finally, it is considered that HTLV-1, which mainly infects CD4+ T lymphocytes, infects the alveolar epithelium and activates NF-κB and AP-1 to cause HTLV-1-related lung disease [17].

It appears that the most effective mechanism by which ANK therapy alleviates HABA is the damage of ATLL cells and activation of the immune system, thereby reducing pulmonary infiltrates and opportunistic infections of ATL cells, which ultimately leads to the improvement of physical findings such as cough, sputum, and dyspnea and the improvement in respiratory functions [18].

CONCLUSION

The two cases presented here and our other treated cases demonstrate that ANK therapy is highly safe and effective for ATL. If further cases can be studied, we believe that there is a good possibility that ANK therapy may become the first-line therapy for ATL instead of chemotherapy and others.

FUTURE PROSPECTS

Low activity of NK cells means accumulation of bacterial load. Therefore, ANK therapy, which administers a large amount of activated NK cells, may be effective not only for ATL and cancer, but also for patients with chronic bacterial

or viral infections. In the future, it is necessary to evaluate these cases extensively.

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