ORIGINAL ARTICLE

Clinicopathological Manifestations and Immune Phenotypes in Adult-Onset Immunodeficiency with Anti-interferon-γ Autoantibodies

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Abstract

Purpose Anti-interferon (IFN)-γ autoantibodies (anti-IFN-γ Abs) is an emerging adult-onset immunodeficiency syndrome. Immune dysfunction in this distinct disorder remains to be clarified.

Methods We prospectively collected blood samples of 20 patients with anti-IFN-γ Abs and 40 healthy normal subjects. The percentages of lymphocyte subpopulations, most relevant to T, B, and NK cells, and the percentages of stimulated lymphocytes with cytokine production were assessed using eight-color flow cytometry. The results were adjusted to age and absolute lymphocyte counts.

Results Most (85%) patients presented nontuberculous mycobacterial infection. Skin lesions were predominantly manifested by neutrophilic dermatoses. The involved lymph nodes had granulomatous inflammation, except 22.2% showing atypical lymphoid hyperplasia without granuloma formation. The percentages of CD4 + T cells and nonactivated subpopulations (recent thymic emigrants and naïve subtypes) decreased significantly with increased expression of activation markers and polarization to differentiated cells. The percentage of NK cells increased, but that of two major NK subpopulations, CD161 + CD56^{bright} and CD161 + CD56 + CD16 + subsets, decreased. Increased CD161^{dim}, CD161 + CD56 − CD16 +, and CD57 + NK cell subsets coupled with the decreased expression of NKp30 and NKp46 indicate reconfiguration of the NK cell population and acquisition of adaptive features. Intracellular cytokine production of the lymphocyte subpopulations was significantly low in the patients compared with the control group.

Conclusion We conclude that the immune system in patients with anti-IFN-γ Abs could be exhausted in T cells and be adaptive in NK cells, contributing to the distinct clinicopathologic features.

Keywords Adult-onset immunodeficiency · Anti-interferon-γ autoantibody · Lymphocyte subpopulations · Cytokine production

Introduction

Adult-onset immunodeficiency due to anti-interferon (IFN)-γ autoantibodies (anti-IFN-γ Abs) is a distinct disorder, recently emerging in Southeast Asia [3, 33]. Thus far more than 600 patients with anti-IFN-γ Abs have been described [18, 30]. Almost all patients are adults aged 30–70 years with no sex predominance [3, 18]. Neutralizing anti-IFN-γ Abs are responsible for the increased susceptibility to infections of nontuberculous mycobacteria (NTM) and intracellular pathogens. The lymph nodes were the most commonly involved organ followed by the skin [13]. Besides direct infections, reactive conditions such as skin manifestation were frequent, with reactive skin disorders, mostly neutrophilic dermatoses, reported in 82% of the patients [14]. Early diagnosis of the disease is difficult owing to the protean manifestations in apparently immunocompetent hosts and the absence of standard laboratory assays [4, 34]. Patients may require long-term antimycobacterial therapy because of chronic infections and high recurrence rates, even after remission [6, 16].

IFN-γ plays a critical regulatory role in macrophage-mediated killing and granuloma formation in response to

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intracellular pathogen infection. Over the past one and a half decades, anti-IFN-γ Abs have been associated with disseminated NTM and other opportunistic infections, including those caused by *Salmonella* species, *Talaromyces marneffei* [12], and varicella-zoster virus [30]. The cause of the production of anti-IFN-γ Abs remains unknown. Genetic factors are strongly suspected to be involved in adult-onset immunodeficiency with anti-IFN-γ Abs. The patients have limited allele polymorphisms of HLA-DRB1 and DQB1 alleles [5, 29]. Titers of anti-IFN-γ Abs may reflect disease activity [16]; however, the pathogenesis of anti-IFN-γ Abs associated with immunodeficiency syndrome remains elusive. A previous study reported that the presence of anti-IFN-γ Abs had immunosuppressive effects in both innate and adaptive immunity. This is done by blocking IFN-γ activated STAT1 activation, IRF1 transactivation, and the production of chemokines and cytokines [17], putting a stop to IFN-γ-induced immunomodulation and antimicrobial activities.

Some studies have highlighted alterations in lymphocyte subpopulations in patients with anti-IFN-γ Abs. The percentages of T cells and naïve subsets decreased, while the number of NK cells increased [3, 7]. Decreased abilities of lymphocyte subpopulations to produce TNF-α, IFN-γ, and IL-2 were reported [17, 32]; however, a comprehensive analysis of the lymphocyte subpopulations and activities is limited. In this study, we have not only described the clinicopathologic features of 20 patients with anti-IFN-γ Abs but analyzed the most relevant T, B, and NK cell subpopulations using eight-color flow cytometry. The percentages of stimulated lymphocyte subsets producing cytokines were evaluated. Our results suggest that the immune dysfunction of adult-onset immunodeficiency due to anti-IFN-γ Abs may be associated with immune exhaustion.

Material and Methods

Study Population

A total of 20 patients diagnosed with anti-IFN-γ Abs at the Chang Gung Memorial Hospital in Kaohsiung, Taiwan, were selected. Anti-IFN-γ Abs in plasma was determined using methods described in supplementary. Absorbance was read at a wavelength of 405 nm (Supplementary Fig. 1). All patients were HIV-negative. To reflect the immune status of patients, we classified the patients into two groups based on their clinical conditions. One group had an active infection or continued antimicrobial therapy within 6 months before the experiments (active cases) and the other recovered from the infection with discontinued therapy (resolved cases). A total of 40 healthy normal subjects with no evidence of immunosuppression were included as a control group. The clinical data of all enrolled patients were collected. The hematoxylin and eosin-stained sections obtained at the time of diagnosis and repeats were reviewed by the pathologist. This study was approved by the Ethics Committee of the Chang Gung Memorial Hospital, Kaohsiung, Taiwan, in accordance with the Declaration of Helsinki (IRB201901509B0C501). Written informed consent was obtained from all subjects prior to enrollment.

Lymphocyte Subpopulations Using Flow Cytometric Analysis

EDTA-anticoagulated whole blood samples were collected for the flow cytometry study. A staining volume of 100 μL (2 to 3×10^6 cells) peripheral blood mononuclear cells (PBMCs) separated using centrifugation was incubated with an antibody cocktail (BD Biosciences, Heidelberg, Germany) (Supplementary Table 1). The eight-color staining panels for the lymphocyte subpopulations were set and analyzed as previously reported [2]. The panels differentiated the subsets from the lymphocyte populations to assess (1) the general lymphocyte overview; (2) B cell subpopulations; (3) CD4 + T cell subpopulations; (4) CD8 + T cell subpopulations; (5) regulatory T cell subpopulations; (6) recent thymic emigrants (RTEs); (7) NK cell subpopulations; and (8) NK cell activation markers. After incubation for 20 min at room temperature (RT, 23 °C) in the dark, the red blood cells (RBCs) were lysed using lysis buffer (BD Biosciences) for 10 min. Following centrifugation and washing with phosphate buffered saline (PBS), the cells were fixed with 200-μL PBS containing 1% formaldehyde and stored at 4 °C in a dark chamber until flow cytometry analysis. We used a FACS Canto II flow cytometer (BD Biosciences) equipped with three lasers (405-nm violet laser, 488-nm blue laser, and 647-nm red laser) for data acquisition and the FACS DIVA software (BD Biosciences) for data analysis.

Cytokine Secretion Assay

The cytokine secretion ability of B, T, and NK cells was detected using flow cytometry. Briefly, we diluted 100 μL of PBMCs with 400 μL of IMDM (Gibco-BRL, Grand Island, NY, USA) in polystyrene tubes and then stimulated the cells with or without the leukocyte activation cocktail (BD GolgiPlug™, Germany) for 4 h at 37 °C in 5% CO₂. After stimulation, fluorochrome-conjugated lineage antibodies (Supplementary Table 1) were added and incubated for 15 min at RT followed by lysis of the RBCs. The cells were then fixed and permeabilized with 250 μL of Cytofix/Cytoperm (BD, Germany) at RT for 20 min. After staining with fluorochrome-conjugated cytokine antibodies (Supplementary Table 1) for 20 min at RT, the cell pellets were washed and resuspended in 200-μL PBS. The FACS Canto II flow cytometer (BD Biosciences) and the FACS DIVA software
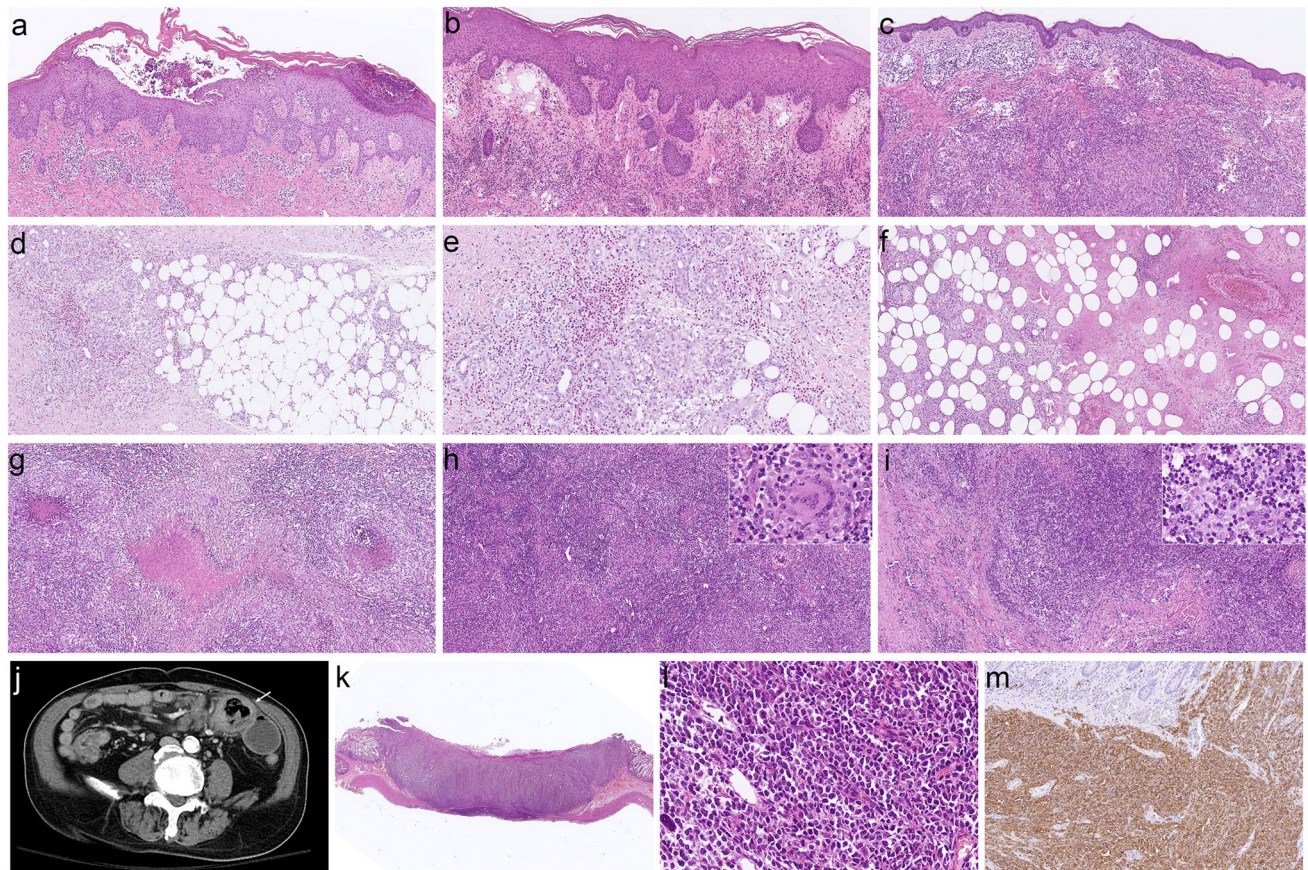
Fig. 1 Histopathologic features in patients with anti-IFN-γ autoantibodies. Representative hematoxylin and eosin (H&E) staining of the skin biopsy shows subcorneal and intraepidermal collection of neutrophils (**a**); a dense neutrophilic infiltration in the dermis (**b**); granulomatous inflammation (**c**); erythema nodosum featuring both septal and lobular infiltrates of lymphocytes, neutrophils, histiocytes, eosinophils, and granulomas with giant cells (**d**, **e**); and erythema induratum characterized by predominant lobular panniculitis with histiocytes forming granulomas, necrosis, and vasculitis (**f**). Histologic features of the lymph node reveal necrotizing granulomatous inflammation (**g**), vague granulomas (**h**), and atypical lymphoid proliferation (inset figure) with thickened capsule (**i**). The computed tomography scan with intravascular contrast enhancement in case 8 shows spiculated wall thickening of the jejunum (arrow) with small bowel obstruction (**j**). The H&E staining demonstrates transmural involvement of the bowel wall (**k**) by infiltrates of large lymphoid cells (**l**), which shows positive CD20 staining (**m**)

(BD Biosciences) were used for data acquisition and analysis, respectively. The gated lymphocyte populations were further assessed for intracellular cytokine-producing cells. The levels of intracellular cytokines after stimulation were quantitatively determined from the percentage of lymphocytes with higher fluorescence intensity than cells without treatment.

Statistical Analysis

All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc. Chicago, IL). The chi-square test, Fisher's exact test, and *t* test were used to compare the data between the two groups. The comparisons between three groups were performed using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) for post hoc comparisons. A two-sided test of significance was used, and $P < 0.05$ was considered statistically significant.

Results

Clinical Features

The mean age was 61.4 years (range: 44.8–82.5 years) in the case group and 53.2 years (range: 25.7–69.9 years) in the control group. The levels of C-reactive protein in the active cases were higher than those in the resolved cases (45.8 ± 18.8 mg/L vs. 12.3 ± 3.9 mg/L, $P = 0.006$). Table 1 summarizes the pertinent clinical presentation. Eighty-five percentage (17/20) of patients had NTM infection, with three patients (3/17, 17.6%) infected by more than one species. The most frequently isolated NTM was *Mycobacterium*

Table 1 Clinicopathologic features of the patients with anti-IFN-γ autoantibodies

Case	Age/sex	Associated infection	Site of involvement	Pattern of dermatosis	Histology of LN/detection of mycobacterium	Antibiotic cessation (m)[a]	Follow-up duration (m)
1	52/F	Disseminated *Mycobacterium abscessus* and *M. chimaera-intracellulare* group infection, *Salmonella* sp. infection, herpes zoster	Blood[b], lung, spine, skin, soft tissue and breast	NA	NA	-	97
2	45/F	Pulmonary *Fonsecaea* sp. infection, *S. choleraesuis* bacteremia, disseminated *M. abscessus* infection	Blood, lymph node, skin, soft tissue, lung and bone	Direct skin and soft tissue infection	NGI/-	-	55
3	71/M	Disseminated *M. chimaera-intracellulare* group infection, herpes zoster	Lung, lymph node, skin and soft tissue	Direct skin and soft tissue infection	LH/-	-	53
4	70/M	*Salmonella* sp. infection, *M. abscessus* lymphadenitis, herpes zoster, *Burkholderia gladioli* bacteremia and deep neck infection	Blood, lung, lymph node, bone, skin and soft tissue	Exanthematous pustulosis	SN/+	+(7)	46
5	45/M	Disseminated tuberculosis, disseminated *M. chimaera-intracellulare* group infection, *S. enterica* serogroup D bacteremia	Blood, lung, bone marrow, spine and soft tissue	NA	LH/-	-	36
6	61/F	Disseminated *M. abscessus* infection	Lymph node and brain	NA	LH/-, SN/+, NGI/+	-	48
7 [6, 21]	65/F	*Salmonella* sp. infection, herpes zoster, disseminated *M. avium* complex infection	Lung, bone, skin, soft tissue, and bone marrow	NA	NA	+(71)	125
8	75/M	*M. hassiacum*, *M. parascrofulaceum*, and *M. farcinogenes-senegalense* infection	Lung, bone, skin, soft tissue, bone marrow and lymph node	Exanthematous pustulosis	NA	+(2)	39
9 [6]	52/F	*S. enterica* serogroup D bacteremia, *M. chimaera-intracellulare* group infection	Blood, nasophargynx, lung, lymph node, skin and soft tissue	Exanthematous pustulosis, direct skin and soft tissue infection	NGI/-	+(11)	89
10 [6]	73/F	Disseminated *M. chimaera-intracellulare* group infection, herpes zoster	Lung, skin, lymph node, spine and bone marrow	NA	ALH/-	+(48)	97
11	62/F	*S. enterica* serogroup D infection, *M. fortuitum* lymphadenitis	Brain and lymph node	NA	NGI/+	+(11)	73
12	48/F	*M. abscessus* and *M. kansasii* infection, herpes zoster	Lymph node, spine and skin	Exanthematous pustulosis, erythema nodosum and erythema induratum	NGI/+	+(12)	65
13	65/M	Disseminated *M. abscessus* infection, herpes zoster	Lymph node, spine and skin	NA	ALH/+, SN/+	-	44
14	58/F	*S. enterica* serogroup B bacteremia, herpes zoster	Blood and skin	NA	ALH/-	-	33
15	60/F	*Salmonella* sp. bacteremia, *M. abscessus* infection	Blood and lymph node	NA	NGI/+	+(6)	11

Table 1 (continued)

Case	Age/sex	Associated infection	Site of involvement	Pattern of dermatosis	Histology of LN/detection of mycobacterium	Antibiotic cessation (m)[a]	Follow-up duration (m)
16	72/M	*M. chimaera-intracellulare* group infection, glucose-nonfermenting gram-negative bacilli infection	Lung, skin and eyes	Sweet's syndrome	NA	+(9)	48
17	56/M	Disseminated *M. abscessus* infection	Nasopharynx, soft tissue and lymph node	Erythema nodosum	NGI/+	-	15
18 [6, 22]	66/M	Disseminated *M. abscessus* infection	Lymph node	Sweet's syndrome	ALH/+, NGI/-	+(51)	90
19	82/F	*S. choleraesuis* bacteremia, *Listeria monocytogenes* infection	Blood and skin	Exanthematous pustulosis	NA	+(6)	13
20	48/F	*Salmonella* sp. infection	Blood and skin	NA	NA	+(1)	3

ALH, atypical lymphoid hyperplasia; m, months; NA, not available; NGI, necrotizing granulomatous inflammation; SN, suppurative necrosis; sp., species

[a]The period between ceasing antimicrobial agents and blood sampling; [b]*M. chimaera-intracellulare* group mycobacteria (The patient had profound immunodeficiency clinically)

Cases 7, 9, 10, and 18 have previously been described. References were cited

abscessus. Case 8 had obstruction and perforation of the jejunum due to a diffuse large B cell lymphoma (DLBCL) (15 months after diagnosis). Skin manifestations were found in 14 patients (70%), including reactive skin conditions and infectious diseases. Cutaneous infection due to *Listeria monocytogenes* was found in Case 19. The skin featured acute onset of generalized edematous erythema with non-follicular sterile pustules.

Pathological Features

Figure 1 depicts the histopathological features of the skin and lymph nodes and the case with malignant lymphoma. Skin biopsies were available in 10 (50%) patients, seven with reactive skin disorders, two (cases 2 and 3) with granulomatous dermatoses, and one (case 9) with reactive lesions followed by granulomatous inflammation. Reactive lesions were mostly neutrophilic dermatoses such as exanthematous pustulosis, Sweet syndrome, and panniculitis. A total of 18 lymph nodes in 14 patients were available. Of these, eight samples (44.4%) showed necrotizing granulomatous inflammation, while three samples (16.7%) showed suppurative necrosis, whereas four (22.2%) samples had atypical lymphoid hyperplasia featuring a histiocytic response without granuloma formation. NTM infection was demonstrated using either acid-fast staining or culture in two patients (cases 13 and 18) with atypical lymphoid hyperplasia. *M. abscessus* was the most frequently isolated species from the lymph nodes rather than from other anatomic locations (7/8, 87.5% vs. 2/9, 22.2%; $P = 0.015$), whereas *M. avium* complex was the predominant species isolated from non-nodal lesions. Case 8 had a diffuse large B-cell lymphoma in the jejunum featuring transmural involvement of the bowel wall by large-sized lymphoma cells.

Distribution of Subpopulations of Peripheral Blood Lymphocytes

The flow cytometry gating strategy and representative immunophenotyping of lymphocyte subsets are shown in Supplementary Fig. 2. Table 2 summarizes the comparison of the lymphocyte subpopulations between the groups based on flow cytometry data. The mean age of the patients was significantly higher than that of the healthy subjects. The percentages of constitutive T and NK cell subsets were significantly different between the case and control groups (Fig. 2). The case groups showed significantly lower levels of CD4 + T cells and nonactivated subpopulations (RTEs and naïve subtypes). We calculated the ratio of RTE/naïve T cells subsequent to the determination of RTEs (CD45RA + CD62L + CD31 +) and naïve T cells (CD45RA + CD62L +) in the same tube. The ratios of CD4 + RTE/CD4 + naïve T cells (0.47 ± 0.12 in the active
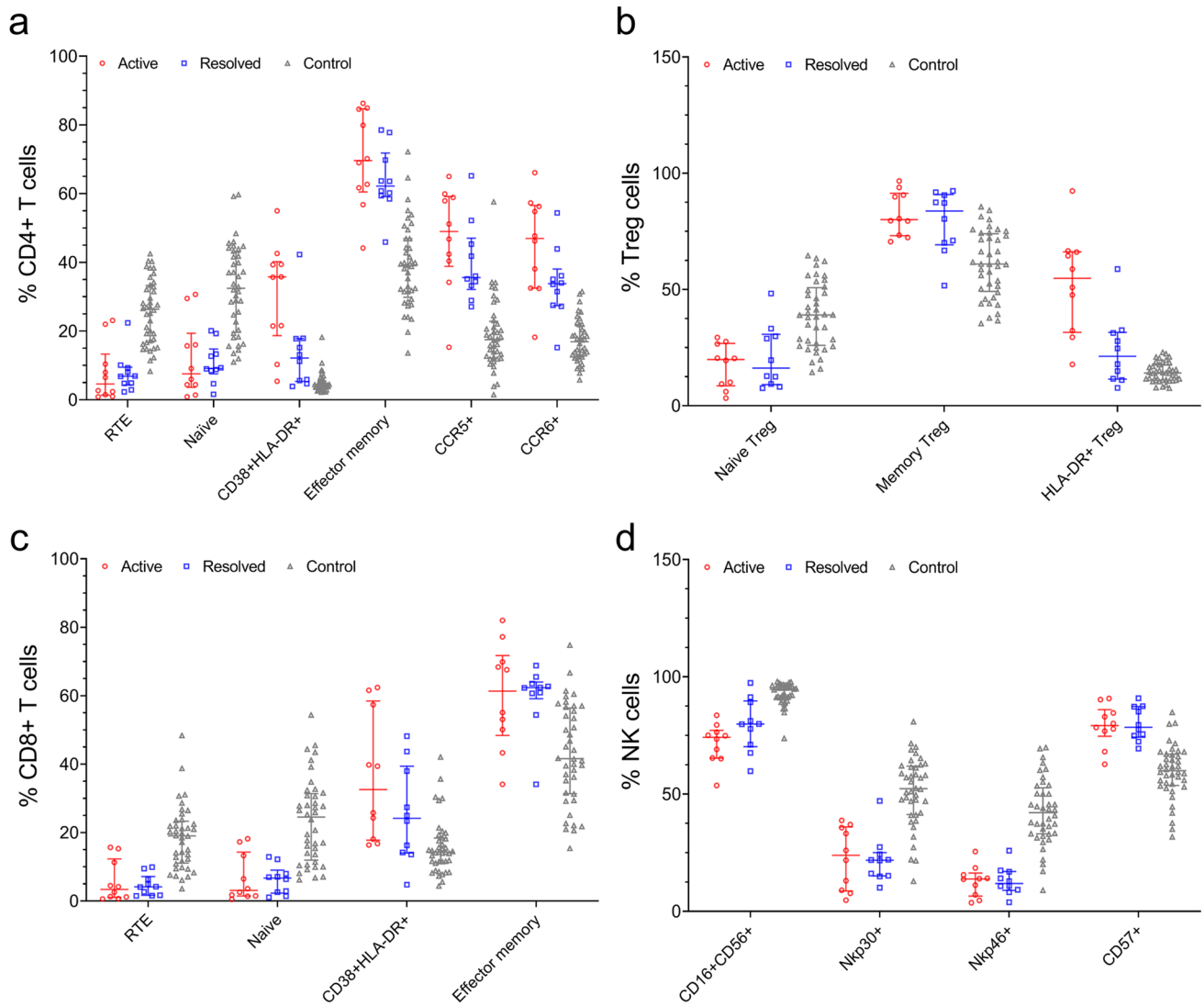
Fig. 2 Comparison of lymphocyte subpopulations between the case and control groups. Dot plots are representative of the percentages of CD4 + T (**a**), Treg (**b**), CD8 + T (**c**), and NK cell subsets (**d**). The lymphocyte subpopulations with a P value < 0.001 between the groups are shown. CCR, C–C chemokine receptor; NK, natural killer; RTE, recent thymic emigrants; TEM, effector memory T cell; Treg, regulatory T

cases; 0.56 ± 0.18 in the resolved cases; 0.62 ± 0.14 in the controls, $P = 0.013$) and CD8 + RTE/CD8 + naïve T cells (0.86 ± 0.14 in the active cases; 0.88 ± 0.09 in the resolved cases; 0.95 ± 0.05 in the controls, $P = 0.006$) were significantly lower in the patients than in the controls. In contrast, those relevant to activated T cell subpopulations showed significant increases in percentages. The NK cell subsets indicated that the case groups had lower levels of NK cells (CD3 − CD16/CD56 +) with the expression of NKp30 and NKp46, while levels of CD57 + NK cells were significantly higher compared to those in the control group. The CD122 and CD161 markers were used to define NK cells in the analysis of NK

cell subpopulations. The percentages of CD161$^{\text{dim}}$ and CD161 + CD56 − CD16 + NK cells were increased in the patients. In addition, significant differences were present between the active and resolved cases. The mean percentages of CD161 + CD56 + CD16 + NK cells (71.6 ± 8.6 vs. 79.5 ± 11.4, $P = 0.012$) and CD4 + TCM (10.9 ± 11.6 vs. 17.4 ± 6.1, $P = 0.026$) were lower; nevertheless, the levels of CD4 + CD38 + HLA-DR + T cells (30.7 ± 15.5 vs. 13.8 ± 11.4, $P < 0.001$), CD4 + CCR6 + T cells (45.0 ± 14.6 vs. 33.9 ± 10.4, $P = 0.007$), CD8 + CD38 + HLA-DR + (36.2 ± 18.7 vs. 25.5 ± 14.1, $P = 0.041$), and HLA-DR + Treg cells (52.7 ± 21.9 vs. 23.8 ± 15.1, $P < 0.001$) were higher in the active cases than in the resolved cases.

Table 2 Lymphocytes subpopulations in the patients with anti-IFN-γ autoantibodies and healthy controls

Variable	Groups			P value
	Active cases (N = 10)	Resolved cases (N = 10)	Controls (N = 40)	
Age (yr)	57.7 ± 10.5	65.1 ± 10.3^a	53.2 ± 8.0^a	0.001
Absolute lymphocyte count ($\times 10^2$ cells/µL)	20.7 ± 7.7	21.9 ± 9.2	20.1 ± 7.6	0.823
T cells (% lymphocytes)	53.7 ± 13.8^b	51.8 ± 12.7^a	65.4 ± 10.2^{ab}	0.001
α/β T cells (% T cells)	93.7 ± 3.8	93.9 ± 4.9	91.0 ± 5.4	0.139
γδ T cells (% T cells)	4.8 ± 4.1	4.8 ± 4.7	8.1 ± 5.3	0.061
CD4 + T cells (% T cells)	23.4 ± 7.9^b	23.2 ± 6.3^a	34.9 ± 7.3^{ab}	<0.001
CD45RA + CD62L + CD31 + (% CD4 + cells)	7.8 ± 8.4^b	7.8 ± 5.7^a	25.6 ± 9.4^{ab}	<0.001
Naïve (% CD4 + cells)	11.8 ± 10.9^b	10.7 ± 5.8^a	32.1 ± 12.9^{ab}	<0.001
CD38 + HLA-DR + (% CD4 + cells)	30.7 ± 15.5^{bc}	13.8 ± 11.4^{ac}	5.0 ± 2.8^{ab}	<0.001
TCM (% CD4 + cells)	10.9 ± 4.2^{bc}	17.4 ± 6.1^c	16.7 ± 6.8^b	0.031
TEM (% CD4 + cells)	70.0 ± 14.0^b	63.8 ± 9.6^a	39.0 ± 12.4^{ab}	<0.001
TE (% CD4 + cells)	4.1 ± 7.7	3.0 ± 1.8	6.3 ± 5.5	0.193
CCR5 + (% CD4 + cells)	47.2 ± 14.9^b	39.9 ± 11.6^a	18.9 ± 10.3^{ab}	<0.001
CCR3 + (% CD4 + cells)	14.9 ± 18.0^b	9.4 ± 13.9	3.0 ± 4.1^b	0.002
CCR6 + (% CD4 + cells)	45.0 ± 14.6^{bc}	33.9 ± 10.4^{ac}	17.1 ± 6.3^{ab}	<0.001
CD8 + T cells (% T cells)	26.4 ± 7.9	24.8 ± 8.4	25.5 ± 7.6	0.893
CD45RA + CD62L + CD31 + (% CD8 + cells)	5.7 ± 6.0^b	4.6 ± 3.1^a	18.5 ± 9.3^{ab}	<0.001
Naïve (% CD8 + cells)	6.8 ± 6.9^b	6.2 ± 4.2^a	23.6 ± 12.4^{ab}	<0.001
CD38 + HLA-DR + (% CD8 + cells)	36.2 ± 18.7^{bc}	25.5 ± 14.1^{ac}	15.8 ± 8.1^{ab}	<0.001
TCM (% CD8 + cells)	4.0 ± 4.8^b	3.8 ± 4.3^a	1.8 ± 1.2^{ab}	0.031
TEM (% CD8 + cells)	60.1 ± 15.4^b	59.5 ± 9.7^a	43.0 ± 14.6^{ab}	<0.001
TE (% CD8 + cells)	21.5 ± 12.0	22.1 ± 11.8	24.5 ± 13.3	0.751
TEM CCR5 + (% CD8 + cells)	37.0 ± 18.5	40.4 ± 9.9^a	28.8 ± 11.2^a	0.017
TE CCR5 + (% CD8 + cells)	9.1 ± 11.7	9.0 ± 5.2	9.7 ± 7.1	0.945
Regulatory T (Treg) cells (% T cells)	6.2 ± 3.3	6.6 ± 4.0	5.0 ± 1.4	0.114
Naïve (% Treg cells)	17.3 ± 9.4^b	21.0 ± 13.6^a	39.0 ± 14.2^{ab}	<0.001
Memory (% Treg cells)	82.7 ± 9.4^b	79.0 ± 13.6^a	61.0 ± 14.2^{ab}	<0.001
HLA-DR + (% Treg cells)	52.7 ± 21.9^{bc}	23.8 ± 15.1^{ac}	14.4 ± 4.2^{ab}	<0.001
B cells (% lymphocytes)	9.3 ± 5.7	9.5 ± 6.6	8.7 ± 3.5	0.849
Transitional (% B cells)	2.7 ± 2.3	2.2 ± 1.8	2.5 ± 1.3	0.783
Naïve (% B cells)	61.8 ± 23.4	61.7 ± 26.2	68.4 ± 13.4	0.398
Non-switched memory (% B cells)	10.7 ± 5.2	8.1 ± 3.3	8.8 ± 5.1	0.448
Switched memory (% B cells)	7.5 ± 7.3	8.5 ± 6.3	12.2 ± 6.1	0.057
CD21^low CD38^low (% B cells)	6.0 ± 5.1	6.8 ± 4.9	5.4 ± 4.4	0.672
Plasmablast (% B cells)	3.4 ± 5.7	1.6 ± 1.4	1.4 ± 0.8	0.076
Plasma cell (% B cells)	3.3 ± 7.3	2.1 ± 2.7	0.8 ± 0.6	0.087
NK cells (% lymphocytes)	36.1 ± 15.7^b	37.9 ± 14.9^a	24.5 ± 10.8^{ab}	0.002
CD161^{dim} (% NK cells)	10.6 ± 7.0^b	13.9 ± 9.1^a	5.8 ± 4.9^{ab}	0.01
CD56 + bright (% CD161 + NK cells)	2.2 ± 1.4^b	2.3 ± 1.1^a	4.2 ± 2.6^{ab}	0.010
CD56 + CD16 + (% CD161 + NK cells)	71.6 ± 8.6^{bc}	79.5 ± 11.4^{ac}	92.8 ± 4.6^{ab}	<0.001
CD56 − CD16 + (% CD161 + NK cells)	21.9 ± 13.9^b	16.6 ± 11.6^a	3.1 ± 3.2^{ab}	<0.001
Nkp30 (% NK cells)	22.7 ± 13.2^b	22.1 ± 10.2^a	51.2 ± 14.8^{ab}	<0.001
Nkp44 (% NK cells)	0.7 ± 0.5	0.6 ± 0.4	0.5 ± 0.4	0.187
Nkp46 (% NK cells)	12.8 ± 6.5^b	13.0 ± 6.1^a	42.3 ± 14.2^{ab}	<0.001
CD57 + (% NK cells)	79.3 ± 8.8^b	80.0 ± 7.3^a	59.7 ± 11.5^{ab}	<0.001

Data presented in means ± standard deviation

Small letters represent statistically significant differences among the groups (ANOVA and LSD)

TCM, central memory T cell; TE, effector T cell; TEM, effector memory T cell

Production of Cytokines in Lymphocyte Subpopulations

We then tested the production of intracellular cytokines of PBMCs that were free of autologous plasma to determine cell-intrinsic functions. Because the levels of intracellular cytokines were very low, we stimulated PBMCs with a polyclonal cell activation mixture containing the phorbol 12-myristate 13-acetate (PMA), ionomycin, and brefeldin A. The cytokines accumulated and localized in the cytoplasm were detected after stimulation. Expressions of TNF-α and IFN-γ were relatively evident in CD4 + (TNF-α, median, 2.2%; range, 0–15.8%; IFN-γ, median, 8.2%; range, 0.7–28.6%) and CD8 + (TNF-α, median, 2.6%; range, 0–14.4%; IFN-γ, median, 11.6%; range, 0.6–32.3%) T cells in the patients. Secretion of IL-2 was restricted in the CD4 + subset (median, 2.8%; range, 0–32.9%). The percentages of TNF-α-producing CD4 + T cells ($P = 0.009$), CD8 + T cells ($P < 0.001$), and NK cells ($P = 0.002$); IFN-γ-producing CD8 + T cells ($P = 0.002$) and NK cells ($P = 0.001$); and IL-2-producing CD4 + T cells ($P < 0.001$) and CD8 + T cells ($P = 0.005$) were significantly lower in the patients compared with the control group. Significant differences between the three groups were seen in the IL-2-producing CD4 + T cells ($P = 0.001$) and TNF-α- ($P = 0.004$), IFN-γ- ($P = 0.010$), and IL-2-producing ($P = 0.018$) CD8 + T cells (Fig. 3).

Discussion

Our data clarify the clinicopathologic and immunological features in patients with anti-IFN-γ Abs. The patients experience recurrent NTM and *Salmonella* infections that are
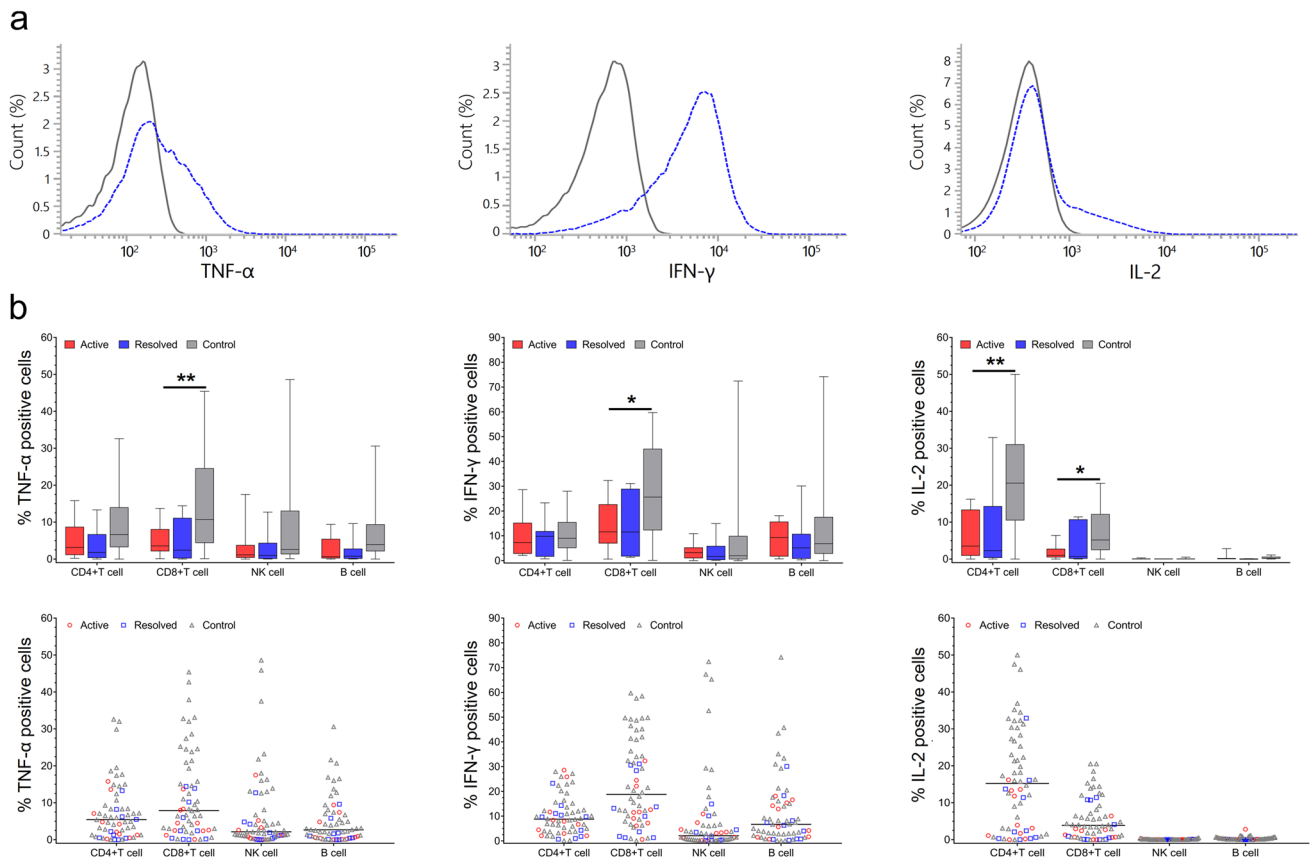

Fig. 3 Intracellular cytokine production in patients with anti-IFN-γ autoantibody and healthy subjects. Peripheral blood mononuclear cells (PBMCs) were stimulated with a leukocyte activation cocktail. The PBMCs were then stained using APC-H7 conjugated anti-CD45 antibody, FITC conjugated anti-CD3 antibody, BV421 conjugated anti-CD4 antibody, APC conjugated anti-CD8 antibody, PE-Cy7 conjugated anti-CD16/anti-CD56 antibodies, PE-conjugated anti-TNF-α antibody, PerCP-Cy5.5 conjugated anti-IFN-γ antibody, and BV510 conjugated anti- IL-2 antibody. The upper panel is representative of figures of the cytokine production in CD8 + T cells (**a**). The increase in the cytokine production in response to the stimulant (blue dash curve) compared to the baseline with no stimulant (gray solid curve) was calculated. The results are represented as a percentage of lymphocytes with the fluorescent intensity higher than that of the baseline (**b**). The comparison data are shown using box plots (upper panel) and dot plots (lower panel). "*" represents $P < 0.05$; "**" represents $P < 0.01$

significantly related to disruption of the IL-12/IFN-γ signaling pathway. Pathogens may enter the thymus through the bloodstream, release pro-inflammatory mediators, and destroy the thymic structure [8]. This has a negative effect on thymopoiesis. The low ratio of RTE to naïve T subsets in the current study might indicate a possible low thymic output, which is highly correlated with the presence of infection. Furthermore, the percentages of NK cells expressing activation markers and cytokine expression in T and NK cells were lower in the patients than those in the control group. It is conceivable that immune exhaustion may exacerbate immune responses to various infectious agents. Additionally, patients may be susceptible to more severe illnesses due to the loss of thymic output and immune exhaustion.

The current understanding of immune dysfunction in patients with anti-IFN-γ Abs is limited. In a study conducted by Browne et al., lymphocyte phenotyping showed a significantly decreased T cell percentage and naïve T lymphocytes, which was consistent with our results [3]. In addition, we found that the percentages of RTEs and naïve T cells decreased and those of activated T cells increased. The dynamic changes in T cell subpopulations raise the question of whether decreased nonactivated T cell subpopulations are age- or disease-related thymic impairments or just a dynamic fluctuation of T cell subsets due to infection. A comparison of the ratio of RTE to naïve T subsets suggested that patients with anti-IFN-γ Abs would have low levels of RTEs in the peripheral T cell pool. This may affect the replenishment and diversity of T cells and result in a delayed regeneration of T cells with a broad TCR repertoire [27]. The resolved cases shared similar immunological parameters with the active cases. It is possible that the resolved cases were still under the effect anti-IFN-γ Abs. Honog et al. reported that the levels of anti-IFN-γ Abs were significantly higher in patients with active infections than in those with resolved infections [13]. Low levels of anti-IFN-γ Abs were persistently present in the patients despite an overall decrease over time. On the other hand, the capacity of the thymus to self-regenerate may be impaired in the resolved cases, indicating decreased thymopoiesis in such cases. The thymus is unable to meet the need to reconstitute an intact thymic function.

An increase in the expression of CD38 + HLA-DR + T cells was detected in the case group. High expression of CD38 + HLA-DR + CD4 + and CD38 + HLA-DR + CD8 + T cell subsets, indicative of T cell activation, is well-known in viral and bacterial infections [11, 20, 31]. However, repetitive or prolonged antigen stimulation may induce the expression of exhaustion markers and decrease IFN-γ production [9]. Wang et al. demonstrated that patients with fatal H7N9 outcomes displayed higher and prolonged expression of CD38 + HLA-DR + on CD8 + T cells than those who survived [31]. The majority of these activated T cells concurrently expressed high

levels of PD-1 with minimal IFN-γ production. In addition, a delay of clonally expanded TCRαβ clonotypes within CD38 + HLA-DR + CD8 + T cells was featured in fatal cases. Similarly, our data revealed high percentages of CD38 + HLA-DR + CD4 + and CD38 + HLA-DR + CD8 + T cells in the patients with anti-IFN-γ Abs. These patients suffered from adult-onset immunodeficiency due to defects in IFN-γ immune surveillance that may lead to recurrent infection. While the status of PD-1 expression was not examined, the decreased expression of intracellular cytokines in the disease group supported exhausted function in these T cells.

In agreement with previous studies, the number of NK cells increased in patients with anti-IFN-γ Abs [3, 7]. Chronic and repeating opportunistic infections may be the predominant cause for the increase in the number of NK cells. We further evaluated subpopulations and functional markers of NK cells. Our data—increased CD161^{dim}, CD161 + CD56 − CD16 +, and CD57 + NK cell subsets coupled with decreased expressions of the activation markers (NKp30 and NKp46) and cytokines (TNF-α and IFN-γ)— indicate reconfiguration of the NK cell population and acquisition of adaptive features. Two major NK subpopulations, CD161 + CD56^{bright} and CD161 + CD56 + CD16 + subsets, were decreased. The CD56 + CD16 + NK cells with a high cytolytic potential are differentiated from the CD56^{bright} NK cells, which proliferate and produce IFN-γ in response to stimulation [1, 26]. However, CD56 − NK cells express higher levels of inhibitory NK receptors and the lower levels of natural cytotoxicity receptors compared to CD56 + NK cells. The CD56 − CD16 + subset was discovered in HIV patients and correlated with cytolytic dysfunction [10]. Likewise, CD57 + cells are defined a subset of highly mature cells in the NK population [24]. The expression of natural cytotoxicity receptors (NKp30 and NKp46) is downregulated during NK-cell maturation [23]. It represents a shift toward a decreased proliferative capacity, low cytokine production, and reduction of cytotoxicity [24, 28]. The accumulation of mature CD57 + NK cell is suggestive of cumulative lifetime exposure to infections, including HIV, hepatitis B and C virus, chikungunya virus, hantavirus, and cytomegalovirus [15]. The decreased expression of CD161 may mark a NK subset with a reduced capacity to proliferative and express IFN-γ and NKp30 in response to pro-inflammatory cytokines [19]. This subset is notably in HIV patients with cytomegalovirus reactivation or reinfection [19]. Altogether, adaptive features of NK cells accentuated by CD161^{dim}, CD161 + CD56 − CD16 +, and CD57 + phenotypes may suggest a consequence of cumulative exposure to infections in the patients with anti-IFN-γ Abs. These NK-cell subsets may have potent lytic activity through CD16-mediated activation [24, 28] and antibody-mediated mechanisms [25]. Further studies are necessary

to clarify the therapeutic strategies to augment NK cells in the adaptive subpopulation.

Studies on the ability of the lymphocyte subpopulations to produce cytokines in patients with anti-IFN-γ Abs are limited. Anti-IFN-γ Abs may block the production of downstream mediators of IFN-γ activity. Krisnawati et al. reported a significant decrease in the levels of serum TNF-α and IFN-γ in all tested serum samples of patients with anti-IFN-γ Abs [17]. The production of IL-2 and TNF-α in CD4 + cells and TNF-α in CD8 + cells was significantly lower in a study conducted by Wipasa et al. [32]. The present study found similar results, with a decrease in cytokine production in the lymphocyte subpopulations. Although cytokine production varied a great deal in the patients, the observation of cytokine responses in resolved cases may account for immune exhaustion and lead to a higher risk of disseminated NTM or other opportunistic infections.

Conclusions

In conclusion, we reported the clinicopathologic features and the immune status of 20 patients with anti-IFN-γ Abs. This study demonstrated that NTM-associated lymphadenitis could develop without granuloma formation and that DLBCL could occur in an unusual site. Additionally, T cells were activated with decreased thymic output, and NK cells were highly matured with decreased expression of activation markers. The lymphocyte subpopulations had a reduction in cytokine responses, which is suggestive of the immune system being exhausted in these patients. The immune dysfunction would decrease immune defense against pathogens and further cause disease exacerbation.

Declarations

Ethics Approval This study was approved by the Ethics Committee of the Chang Gung Memorial Hospital, Kaohsiung, Taiwan, in accordance with the Declaration of Helsinki (IRB201901509B0C501).

Consent to Participate All participants provided written informed consent.

Consent for Publication Obtained.

Conflict of Interest The authors declare no competing interests.

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