

# CD56+ T cells in the peripheral blood of uveitis patients

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## Abstract

**Aims**—Natural killer T (NKT) cells, T lymphocytes expressing both T cell and NK cell markers, are suggested to be involved in autoimmune diseases. To examine the relation between the pathogenesis of uveitis and CD56+ T cells, which are thought to be a type of human NKT cells, we investigated peripheral CD56+ T cells in uveitis patients.

**Methods**—41 uveitis patients (Behçet's disease (BD), 14; sarcoidosis (SAR), eight; Vogt-Koyanagi-Harada disease (VKH), five; idiopathic uveitis (IU), nine; and others, five) and 19 healthy controls participated in this study. Cell surface antigens of lymphocytes were analysed by use of monoclonal antibodies and flow cytometry.

**Results**—The proportion of CD56+ T cells in patients with BD was higher than in controls and in patients with SAR, VKH, IU, and others.

**Conclusion**—Increased peripheral CD56+ T cells might be relevant to the pathogenesis of uveitis in BD, and increase of peripheral CD56+ T cells may be one of the laboratory findings to suggest that uveitis originates from BD.

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Uveitis is an inflammation of the uveal tract. Although its immunopathogenesis remains to be elucidated, substantial evidence indicates that alteration of T cell mediated responses is involved.<sup>1,2</sup> In Japan, uveitis often appears as a manifestation of a systemic disease associated with immunological abnormalities, such as Behçet's disease (BD), sarcoidosis (SAR), and Vogt-Koyanagi-Harada disease (VKH).<sup>2</sup>

Uveitis in BD often exhibits serious clinical features compared with other forms of uveitis.<sup>3</sup> Several studies have suggested that  $\gamma\delta$  T cells, which are known to be extrathymic T cells,<sup>4</sup> might play an important role in the pathogenesis of BD.<sup>5,6</sup>

Attention has recently focused on natural killer T (NKT) cells, T lymphocytes expressing both T cell and NK cell markers,<sup>7</sup> which are also thought to be extrathymic T cells.<sup>8</sup> It was reported that CD56+ T cells were one population of human extrathymic T cells, and contained large proportion of  $\gamma\delta$  T cells.<sup>9,10</sup> CD56+ T cells are thought to be associated with autoimmune diseases.<sup>11,12</sup>

In the present study to examine the involvement of CD56+ T cells in the pathogenesis of uveitis, CD56+ T cells were investigated in the peripheral blood of uveitis patients.

## Patients and methods

### PATIENTS

Forty one patients with uveitis and 19 healthy controls participated in this study. All uveitis patients received steroid eye drops at the time of blood sampling. Patient characteristics are summarised in Table 1. In 14 BD patients, five were the complete type and nine were the incomplete type according to the criteria of the Behçet's Disease Research Committee in Japan.<sup>13</sup> In 27 uveitis patients without BD, diagnoses were based on detailed history, ocular examinations, and laboratory tests. Informed consent was obtained from all patients and healthy controls.

### METHODS

Heparinised venous blood was obtained from uveitis patients and healthy controls. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation at 400 *g* on a Ficoll-Paque (Pharmacia) gradient. PBMC were recovered at the interface and washed. Monoclonal antibodies (mAbs) used for labelling assays were all purchased: FITC conjugated anti-TCR  $\gamma\delta$  (Becton Dickinson), PE conjugated anti-CD56 (Pharmingen), and PE-Cy5 conjugated anti-CD3 (Coulter Corp). Flow cytometry analysis was performed on an Epics flow cytometer (Coulter Corp). Freshly isolated cells were incubated in cold HBSS with appropriate dilutions of these mAbs for two colour staining. A gate for the live lymphocyte population was defined by forward and side scatter characteristics, and data were displayed on a log scale of increasing fluorescence intensity. Data were analysed by one way analysis of variance with Student's *t* test.

## Results and discussion

$\gamma\delta$  T cells were suggested to play an important role in the pathogenesis of BD.<sup>5,6</sup> We examined the proportion of peripheral  $\gamma\delta$  TCR+ cells in CD3+ cells in uveitis patients with BD, uveitis patients without BD, and controls. The proportion of  $\gamma\delta$  TCR+ cells in CD3+ cells in uveitis patients with BD were significantly higher than those in controls (7.4 (SD 8.8) *v* 2.8 (1.5), *p* < 0.01), but were not significantly higher than those in uveitis patients without BD (7.4 (8.8) *v* 4.0 (3.5), *p* = 0.10). The results corresponded with the previous data.<sup>6</sup>

Next, we examined the proportion of CD3+CD56+ cells in CD3+ cells in controls and in patients with BD, SAR, VKH, IU and others (Fig 1). In patients with BD the proportion of CD3+CD56+ cells in CD3+ cells was significantly higher (10.8 (8.6)) than in controls (2.5 (1.5)) and patients with SAR

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Table 1 Patient characteristics

	Behçet's disease	Sarcoidosis	Vögt-Koyanagi-Harada disease	Idiopathic uveitis	Others*	Controls
No of patients	14	8	5	9	5	19
Age (year) (mean (SD))	47.5 (15.3)	63.0 (7.5)	44.6 (17.3)	44.2 (17.3)	47.2 (18.4)	50.2 (15.2)
Range	20–71	52–72	20–62	21–69	24–74	19–70
Sex (M/F)	14/2	1/7	4/1	1/8	3/2	11/8
Extraocular manifestations	3 (mucocutaneous) 1 (neurological)	2 (lung)	0	0	0	
Active uveitis/inactive	7/7	6/2	4/1	6/3	5/0	
Anterior/posterior	4/3	3/3	2/2	3/3	3/2	
Medication (oral agents)						
Prednisolone ( $\leq 15$ mg/day)	2	2	5	2	1	
Cyclosporine ( $\leq 5$ mg/kg/day)	8					
Colchicine	5					
Mizoribine	1					
Aciclovir					2	

\*Others include the following: acute retinal necrosis, n=2; acute anterior uveitis, n=1; Posner-Schlossman syndrome, n=1; iritis associated with diabetes mellitus, n=1.

(3.0 (3.3)), VKH (2.7 (2.0)), IU (2.5 (1.1)), and others (1.6 (1.2)). CD56+ T cells increased only in uveitis patients with BD. Further, no significant difference was found in the proportion of CD56+ T cells in BD patients with active or inactive uveitis (data not shown).

Murine NKT cells, lymphocytes uniquely expressing both TCR/CD3 and NK1.1+ antigens, were discovered in thymus.<sup>7</sup> NKT cells in mice preferentially use invariant V $\alpha$ 14 gene products for their T cell antigen receptor (TCR), and mouse V $\alpha$ 14 and human V $\alpha$ 24 have a sequence homology.<sup>8–14</sup> Since V $\alpha$ 24+ double negative (DN) T cells in human express NKR-P1 antigen, which is one of the NK markers, human NKT cells are suggested to coincide with V $\alpha$ 24+ DNT cells.<sup>15</sup> Selective reduction of V $\alpha$ 24+ DNT cells was reported in patients with systemic sclerosis and insulin dependent diabetes mellitus.<sup>11–12</sup>

On the other hand, it was reported that human CD56+ T cells have similar characteristics to murine NK1.1+ T cells—namely, they are large granular lymphocytes, morphologically like NK cells, and contain a significant proportion of CD4–8– cells or  $\gamma\delta$  T cells.<sup>9–10</sup> More importantly, they are abundant in the liver, where extrathymic T cells could differentiate.<sup>9–10</sup> These characteristics raised the possibility that CD56+ T cells are a counterpart of extrathymic T cells in humans. Such

unique T cells increased in the presence of malignancies,<sup>9</sup> with aging,<sup>16</sup> and in tumour tissues.<sup>9</sup> We found that the proportion of CD56+ T cells was increased significantly in uveitis patients with BD compared with the other uveitis patients, indicating that CD56+ T cells might be relevant to uveitis with BD. Since murine NK1.1+ T cells produce both IFN- $\gamma$  (T helper 1 (Th1) cytokine) and IL-4 (Th2 cytokine) and regulate Th1/Th2 differentiation,<sup>17</sup> CD56+ T cells may play a part in the differentiation from naive T cells into Th1 or Th2 cells. Th1 cells are known to be enriched in the peripheral blood from BD patients.<sup>18</sup> Interleukin-2 (IL-2), which is released by the increased Th1 cells, may induce proliferation of CD56+ T cells and enhance their cytotoxic effects.<sup>19</sup> Also, CD56+ T cells may recognise heat shock protein (HSP) and proliferate in the same way as  $\gamma\delta$  T cells.<sup>20</sup> Together with  $\gamma\delta$  T cells, CD56+ T cells may contribute to tissue damage in BD through their enhanced cytotoxic effects.<sup>5–19</sup> Alternatively, in the same way that CD57+ T cells, which are another subset of human NKT cells, suppress the inflammation of rheumatoid arthritis,<sup>21</sup> CD56+ T cells also may inhibit the inflammation of BD.

Recently, it was reported that IL-12, which could activate CD56+ T cells and augment their cytotoxicity in the presence of IL-2,<sup>19</sup> increased in the aqueous humour (AH) and vitreous of patients with uveitis.<sup>22</sup> In fact, we have found that AH from active uveitis patients with BD includes high proportion of CD56+ T cells (Yato *et al*, unpublished data). These results suggested that an increase of peripheral CD56+ T cells might be related to activity of uveitis. Unexpectedly, we found no significant difference in CD56+ T cells in peripheral blood between active uveitis patients with BD and inactive ones. It does not seem reasonable to evaluate the activity of uveitis with BD, which frequently exhibits other focal lesions, by analysis of peripheral CD56+ T cell proportion. Further investigation of larger series will define the relation between CD56+ T cells and the severity or locality.

In conclusion, the proportion of peripheral CD56+ T cells was increased in uveitis patients with BD, and not in other uveitis patients. This suggested that CD56+ T cells were involved in uveitis with BD in the same

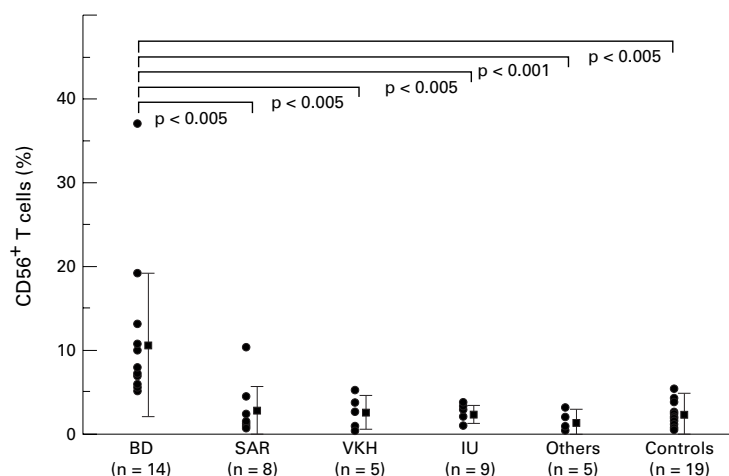


Figure 1 Proportion of CD3+CD56+ cells in CD3+ cells in controls and patients with Behçet's disease (BD), sarcoidosis (SAR), Vögt-Koyanagi-Harada disease (VKH), idiopathic uveitis (IU), and others. Solid circles represent individual data points and solid squares and bars represent population mean (SD).

way as  $\gamma\delta$  T cells. Since Japanese BD patients frequently suffer recurrent, severe attacks of uveitis during systemic corticosteroid therapy,<sup>23</sup> which is the first course of general uveitis treatment, we have to determine promptly whether the uveitis originates from BD or not. The increase of peripheral CD56+ T cells may be one of the laboratory findings to suggest that the uveitis originates from BD.

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