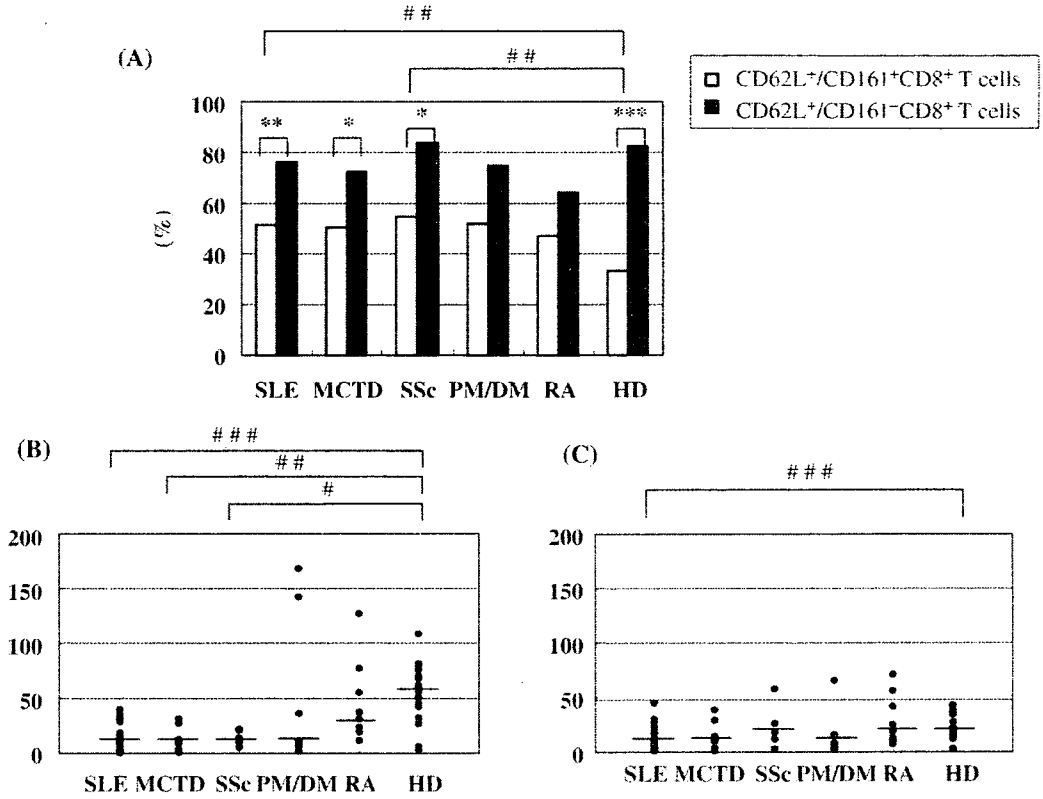

FIG. 4. (A) Frequency of CD62L expression on $CD161^+CD8^+$ T cells and $CD161^-CD8^+$ T cells in the peripheral blood of patients suffering from rheumatic diseases (%). The frequency of CD62L expression on $CD161^+CD8^+$ T cells in the peripheral blood (clear bars) was significantly decreased in SLE, MCTD, SSc and the healthy donors when compared with $CD161^-CD8^+$ T cells (solid bars). The frequency of CD62L expression on $CD161^+CD8^+$ T cells was significantly increased in SLE and SSc when compared with the healthy donors (HD). The mean ± S.D. of the frequency of CD62L expression on $CD161^+CD8^+$ T cells was 55.1 ± 20.4 in patients with SLE, 51.1 ± 23.9 in patients with MCTD, 54.0 ± 22.1 in patients with SSc, 52.1 ± 25.4 in patients with PM/DM, 52.7 ± 24.2 in patients with RA, and 34.4 ± 13.5 in the healthy donors. The mean ± S.D. of the frequency of CD62L expression on $CD161^-$ $CD8^+$ T cells were 73.8 ± 18.8 in patients with SLE, 72.8 ± 21.3 in patients with MCTD, 82.9 ± 3.7 in patients with SSc, 74.8 ± 19.8 in patients with PM/DM, 67.9 ± 25.1 in patients with RA and 83.0 ± 9.9 in the healthy donors, respectively. All bars indicate the mean values. (B) Absolute numbers of $CD161^+CD8^+CD62L^-$ T cells in the peripheral blood (cell count/μl of whole blood). Absolute numbers of $CD161^+CD8^+CD62L^-$ T cells in the peripheral blood were significantly decreased in patients with SLE, MCTD and SSc. The median (range) of the absolute number of these cells was 9 (0–40) in patients with SLE, 11 (0–31) in patients with MCTD, 14 (5–22) in patients with SSc, 11 (2–168) in patients with PM/DM, 31 (11–127) in patients with RA and 57.5 (3–108) in the healthy donors. Horizontal bars indicate the median. (C) Absolute numbers of $CD161^+CD8^+CD62L^+$ T cells in the peripheral blood (cell count/μl of whole blood). Absolute numbers of $CD161^+CD8^+CD62L^+$ T cells in the peripheral blood were significantly decreased only in patients with SLE. The median (range) of the absolute number of these cells was 7.5 (1–44) in patients with SLE, 10 (1–39) in patients with MCTD, 18 (3–58) in patients with SSc, 7.5 (2–65) in patients with PM/DM, 19 (7–71) in patients with RA and 20 (3–43) in the healthy donors. Horizontal bars indicate the median. SLE, $n = 26$; MCTD, $n = 10$; SSc, $n = 6$; PM/DM, $n = 8$; RA, $n = 11$; HD, $n = 18$. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

Discussion

The present study is the first to demonstrate a decrease of $CD161^+CD8^+$ T cells in the peripheral blood of patients suffering from SLE, MCTD, SSc and PM/DM. This interesting finding is of considerable significance, since there has been little information concerning $CD8^+$ NKT cells in autoimmune diseases. With regard to human NKT cells, it has been reported that both $CD4^-CD8^-$ and $CD4^-$ NKT cells are generally decreased in patients with SLE, SSc, RA and SS [4], suggesting that these cells play a role in the pathogenesis. Therefore, the results of the present study suggest that $CD161^+CD8^+$ T cells, which are closely related to $CD8^+$ NKT cells, also contribute specifically to the pathogenesis of rheumatic diseases, as well as $CD4^-CD8^-$ and $CD4^+$ NKT cells.

On the other hand, the common contribution of $CD4^-CD8^-$ and $CD4^+$ NKT cells to pathogenesis is their regulatory function. Indeed, a previous study using a mouse model has suggested that these NKT cells may act as regulatory T cells, and that a reduction in their number is associated with the occurrence of autoimmune diseases [4]. A recent investigation has also suggested that $CD4^+$ NKT cells are regulators of not only tumour immunity, but also of autoimmune diseases in humans [20]. Furthermore, a recent study has shown that $CD8^+$ NKT cells may exert a regulatory role in humans through lysis of APCs or activated T cells [9]. Thus, it is possible that $CD161^+CD8^+$ T cells, like $CD8^+$ NKT cells, may prevent autoimmunity. Indeed, in the present study, the decrease of $CD161^+CD8^+$ T cells observed in SLE, MCTD and SSc included a high proportion of $CD28^-CD8^+$ T cells. Therefore, suppressor T cells may also have been depleted.

The observations suggest that the decrease of of $CD161^+CD8^+$ T cells may also play a role in the pathogenesis of rheumatic diseases though the disruption of the regulatory mechanism associated with the decrease of other populations of NKT cells. However, since the decrease in $CD161^+CD8^+$ T cells was not related to disease activity or to other populations of NKT cells, it may play only a partial role in the development of rheumatic diseases.

Another point of interest is whether the CD161 molecule has a regulatory function. We have demonstrated that the CD161 molecule is expressed more frequently on $CD4^+CD25^+$ T cells than on $CD4^+CD25^-$ T cells, suggesting that it may play a regulatory role. However, $CD4^+CD25^+$ T cells do not represent a homogeneous population of regulatory cells, because CD25 is also expressed diffusely on activated effector T cells. Therefore, it may be necessary to investigate the frequency of CD161 expression on $Foxp3^+CD4^+CD25^+$ T cells. In addition, anti-CD161 blocking antibody did not enhance the production of IgG upon stimulation with PWM. Therefore, clearer evidence for a regulatory function of CD161 will be needed. Since anti-CD161 antibody possessing an enhancing function is not commercially available, we are unable to investigate other effects such as the decrease of antibody generation. If anti-CD161 antibody with an enhancing function becomes available in the future, we would certainly be interested in investigating other effects.

On the other hand, there was no reduction in the frequency of CD161 expression on $CD8^+$ T cells in RA patients, suggesting that the pathogenesis of RA may differ from that of other rheumatic diseases. Our previous study has shown that the expression of NK-B1 on $CD8^+$ T cells is decreased only in RA patients, suggesting a specific mechanism for the activation of $CD8^+$ T cells in this disease [21]. Therefore, the absence of any reduction in the frequency of CD161 expression on $CD8^+$ T cells in RA may reflect the fact that the characteristics of the $CD8^+$ T cells involved differ according to the type of disease.

Although we also examined the expression of CD161 on $CD4^+$ T cells, the absolute number of $CD161^+CD4^+$ T cells was significantly decreased only in SLE patients. It is likely that this decrease is a consequence of the decrease in $CD4^+$ T cells commonly observed in SLE patients. In addition, there was also no difference in the expression of CD161 on $CD4^+CD25^+$ T cells. Therefore, our results suggest that there may be no essential difference in $CD161^+CD4^+$ T cells, including $CD4^+$ NKT cells, between patients with rheumatic diseases and healthy donors. However, this result differs from that of another study [4], and the discrepancy may be related to the fine difference in cell population between $CD4^+CD161^+$ T cells and $CD4^+$ NKT cells. Thus, our present study demonstrated abnormal CD161 expression only in $CD8^+$ T cells, and not in $CD4^+$ T cells.

Finally, we consider the reason for the decrease of $CD161^+CD8^+$ T cells. Our observations on the regulation of CD62L provide an important clue. In brief, the main population of $CD161^+CD8^+$ T cells was CD62L-negative in healthy donors, and this was the main cell component that was decreased in patients with rheumatic diseases. The former observation is in agreement with a report that $CD161^+CD8^+$ T cells are present in extra-thymic or extra-lymphoid tissues [22], since CD62L-negative cells are localized mainly in tissue and peripheral blood. The latter observation provides some clue as to the cause of the decrease in these cells. First, it is possible that $CD161^+CD8^+CD62L^-$ T cells migrated into the inflammatory tissue. However, the observation that the decrease in $CD161^+CD8^+$ T cells was not related to disease activity, and a report that $CD4^+CD62L^+$ T cells migrate into inflammatory tissue [23], argue against this possibility. In addition, it is difficult to collect many samples of inflamed tissue from humans, even though direct observation of inflamed tissue for infiltration of $CD161^+CD8^+$ T cells would be desirable in order to investigate the tissue-homing properties of these cells. Second, it is possible that the production of $CD161^+CD8^+$ T cells in the bone marrow or extra-thymic/lymphoid tissues may be decreased. Most studies [24, 25] have found that CD62L-negative cells are derived from inflammatory tissue, although one study has demonstrated that naive T cells lack CD62L expression in human neonates [26]. Therefore, since it is believed that $CD161^+CD8^+CD62L^-$ T cells develop mainly in extra-thymic/lymphoid tissues, production of this T cell population at these sites may be suppressed by some mechanism, although this is difficult to prove. Third, it is possible that $CD161^+CD8^+$ T cells are decreased by apoptosis. However, since no significant correlation could be demonstrated between the absolute number of $CD161^+CD8^+$ T cells and that of apoptotic $CD8^+$ T cells expressing Annexin V^+/propidium iodide$^-$ (data not shown), we think this possibility is unlikely. Moreover, inadequate antigen presentation, dysfunction of $CD8^+$ NKT cells, and abnormal antigen presentation [4] may be partly involved in the decrease of $CD161^+CD8^+$ T cells, as is the case for $CD4^-CD8^-$ and $CD4^+$ NKT cells. Moreover, a possible relationship between $CD8^+$ NKT cells and infection, for example HIV or previous vaccination, cannot be ignored. However, a previous report has denied this possibility [27], and none of the patients included in the present study were injected with vaccines in a month prior to our analysis. A number of aspects concerning the role and origin of $CD161^+CD8^+$ T cells remain unclarified, and further studies will therefore be required.

In summary, we have demonstrated that both the frequency and the absolute number of $CD161^+CD8^+$ T cells are decreased in the peripheral blood of patients suffering from SLE, MCTD, SSc and PM/DM. We are convinced that our present findings provide a new clue to the etiology of these disorders.

Rheumatology	Key message
	• Both the frequency and the absolute number of $CD161^+CD8^+$ T cells were decreased in the peripheral blood of patients suffering from SLE, MCTD, SSc and PM/DM.

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