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Differential Expression of CD11c by Peripheral Blood NK Cells Reflects Temporal Activity of Multiple Sclerosis

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Multiple sclerosis (MS) is an autoimmune disease, showing a great degree of variance in temporal disease activity. We have recently demonstrated that peripheral blood NK cells biased for secreting IL-5 (NK2 bias) are associated with the remission state of MS. In this study, we report that MS patients in remission differentially express CD11c on NK cell surface (operationally defined as CD11c$^{high}$ or CD11c$^{low}$). When we compared CD11c$^{high}$ or CD11c$^{low}$ patients, the expression of IL-5 and GATA-3 in NK cells supposed to endow a disease-protective NK2 phenotype was observed in CD11c$^{high}$ but not in CD11c$^{high}$ patients. In contrast, the CD11c$^{high}$ group showed a higher expression of HLA-DR on NK cells. In vitro studies demonstrated that NK cell stimulatory cytokines such as IL-15 would up-regulate CD11c expression on NK cells. Given previous evidence showing an association between an increased level of proinflammatory cytokines and temporal disease activity in MS, we postulate that inflammatory signals may play a role in inducing the CD11c$^{high}$ NK cell phenotype. Follow-up of a new cohort of patients showed that 6 of 10 CD11c$^{high}$ MS patients developed a clinical relapse within 120 days after evaluation, whereas only 2 of 13 CD11c$^{low}$ developed exacerbated disease ($p = 0.003$). As such, a higher expression of CD11c on NK cells may reflect the temporal activity of MS as well as a loss of regulatory NK2 phenotype, which may allow us to use it as a potential biomarker to monitor the immunological status of MS patients. The Journal of Immunology, 2006, 177: 5659–5667.
CD11chigh NK cells. In contrast, expression of HLA-DR class II molecule was up-regulated in CD11chigh NK cells. Notably, both CD11c and HLA-DR on NK cells were reproducibly induced in vitro in the presence of IL-15 (11) or combination of inflammatory cytokines, known to be increased in the blood of MS (12–14). Furthermore, we found that the remission state of CD11chigh is unstable in comparison to CD11clow, as judged by an increased number of the patients who exacerbated during the 120 days after examining NK cell phenotypes. These results suggest that the CD11chigh group of patients may be in more unstable condition than CD11clow, presenting with reduced regulatory functions of NK cells.

Materials and Methods

Subjects

Twenty-five patients with relapsing-remitting MS (15) (male (M)/female (F) = 8/17, age = 37.7 ± 11.1 (year old) and 10 sex- and age-matched HS (M/F = 37; age = 39.9 ± 12.2 (year old)) were enrolled for studying NK cell phenotypes. All the patients were in the state of remission at examination as judged by magnetic resonance imaging scanning and clinical assessment. They had not been given immunosuppressive medications, or corticosteroids for at least 1 mo before examination. They had relatively assessment. They had not been given immunosuppressive medications, or corticosteroids without knowing any information on the NK cell phenotype, the patient was considered as the dropout at that time point. Remission rate was calculated as Kaplan-Meier survival rate, and statistical difference between CD11chigh and CD11clow MS was evaluated with the log-rank test.

Results

CD11c on NK cells is up-regulated in MS remission

First, we confirmed that PBMC from healthy individuals and MS contain CD11c+ NK cells (Fig. 1), which constitute a major population of whole NK cells. We then noticed that proportion of CD11c+ NK cells as well as its levels of expression greatly varied among individuals, particularly in MS. To examine this issue further, we systematically examined 25 MS patients in remission and 10 HS for NK cell expression of CD11c. Whereas 20–80% of NK cells are CD11c+ in HS (Fig. 1c), almost all NK cells were CD11c+ in some MS patients (Fig. 1, c and e). However, reflecting a great degree of variance, comparison between HS and MS did not reveal a significant difference (Fig. 1c). In contrast, when we measured the MFI of CD11c expression on CD11c+ NK cells, it was significantly higher in MS as compared with HS (Fig. 1a). This difference was also noticed when MFI of CD11c was measured for all the NK cell populations (Fig. 1b). It was interesting to know whether the levels of CD11c expression may correlate with NK cell functions. Therefore, we operationally divided the MS patients into CD11clow and CD11chigh subgroups (Fig. 1a), by setting the border as (the average + 2 × SD) of the values for HS.

CD11chigh NK cells express HLA-DR more brightly than CD11clow NK cells

It was previously reported that infection with certain viruses would accompany up-regulation of CD11c on NK cells (16). This raises a possibility that the increased expression of CD11c in CD11chigh MS may reflect an activation state of NK cells caused by some sort of stimuli. To verify this hypothesis, we examined surface expression of cell activation markers (CD69 and HLA-DR). Although CD69, an early activation marker, was not detectable on NK cells (Fig. 2a), NK cells from MS, particularly CD11chigh MS, significantly overexpressed HLA-DR on surface (Fig. 2). Interestingly, HLA-DR expression was also up-regulated on CD4+ T cells from CD11chigh MS compared with those from HS (data not shown). These results indicate that NK cells and T cells are differentially activated in CD11chigh MS, CD11clow MS, and HS.

Absence of NK2 bias in CD11chigh MS

We have previously reported that a higher level of IL-5 expression (NK2 bias) is one of the characteristics of NK cells in MS

RT-PCR

Total RNA were extracted with a RNeasy Mini kit (Qiagen) from purified NK cells, and the cDNA were synthesized with Super Script III first strand systems (Invitrogen Life Technologies) according to the manufacturer’s protocol. For quantitative analysis of IL-5, IFN-γ, GATA-3, and T-bet, the LightCycler quantitative PCR system (Roche Diagnostics) was used. Relative quantities of mRNA were evaluated after normalizing each expression level with β-actin expression. PCR primers used were as follows: β-actin-sense, AGAGATTGGCCACGGTGCTTT, and -antisense, ATTT GCGGTTGACGATGGG; IFN-γ-sense, CAGTTCCCCACAGTGA GCC, and -antisense, GCTTTGGTCATCTCG; IL-5-sense, GCA CACTGGAGATGCAACT, and -antisense, CACTCGGTGTTCA TACC; GATA-3-sense, CTACGGAAACTCGGTCAGG, and -antisense, CTGTTAATTTGAGCACTCTT; T-bet-sense, GGAGGACACCGACTA ATTTGGGA, and -antisense, AAGCAGAGCACCGCACAGTTAA.

Statistical analysis of remission rate

We set the first episode of relapse after blood sampling as an end point, although we followed clinical course of each patient for up to 120 days, regardless of whether they developed relapses. No patients developed second relapse during the 120 days. When the neurologist prescribed corticosteroids without knowing any information on the NK cell phenotype, the patient was considered as the dropout at that time point. Remission rate was calculated as Kaplan-Meier survival rate, and statistical difference between CD11chigh and CD11clow MS was evaluated with the log-rank test.

Reagents

Mouse IgG1 isotype control-PE, anti-CD3-energy-coupled dye (ECD), anti-CD4-PE, anti-CD8-PC5, anti-CD56-PC5, anti-CD69-PE, and anti-HLA-DR-FITC mAbs were purchased from Immunotech. Anti-CD11c-PE and anti-CD95-FITC were purchased from BD Pharmingen. Recombinant human cytokines were purchased from PeproTech. AIM-V (Invitrogen Life Technologies) was used for cell culture after supplementing 2 mM t-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen Life Technologies).

Cell preparation and NK cell purification

PBMC were separated by density gradient centrifugation with Ficoll-Hypaque PLUS (Amersham Biosciences). To purify NK cells, PBMC were treated with NK isolation kit II (Miltenyi Biotec) twice, according to the manufacturer’s protocol. Briefly, PBMC were labeled with a mixture of biotin-conjugated mAbs reactive to non-NK cells and magnetic microbead-conjugated anti-biotin mAbs. The magnetically labeled non-NK cells were depleted with auto-MACS (Miltenyi Biotec) and this procedure always yielded >95% purity of NK cells when assessed by the proportions of CD3+ CD56+ cells with flow cytometry.

Flow cytometry

To evaluate the expression of CD11c, CD95, or other surface molecules on NK cells, PBMC were stained with anti-CD3-ECD, anti-CD56-PC5, and FITC- or PE-conjugated mAbs against molecules of our interest and were analyzed with EPICS flow cytometry (Beckman Coulter). Mean fluorescence intensity (MFI) of CD11c was measured on gated CD11c+ fraction or whole NK cells.

Stimulation of purified NK cells with proinflammatory cytokines

Purified NK cells (1 × 10^6/well) were stimulated in the presence or absence of IL-4, IL-8, IL-12, IL-15, IL-18, IL-23, TNF-α, and GM-CSF or combination of IL-12, IL-15, and IL-18 for 3 days. We analyzed CD11c expression after staining the cells with anti-CD11c-PE, anti-CD3-ECD, and anti-CD56-PC5. The concentration of IL-12 was at 10 ng/ml, and those of the other cytokines were at 100 ng/ml.
remission (3). Although the mechanism for NK2 bias in MS remains to be further studied, up-regulation of GATA-3 has recently been reported in the induction of NK2 cells in mice (17). To explore the possible difference in the functions of CD11chigh and CD11clow NK cells, we isolated NK cells from CD11c high or CD11clow group of patients and measured the mRNA levels of representative cytokines IFN-γ and IL-5 as well as corresponding transcription factors T-bet and GATA-3. As shown in Fig. 3, mRNA expression of both IL-5 and GATA-3 was significantly higher in CD11clow MS compared with HS or CD11chigh MS, indicating that NK2 bias thought to be characteristic of MS remission is restricted to CD11clow MS. In contrast, there were no differences in mRNA expression of IFN-γ and T-bet among these three groups. Because NK cells from CD11chigh patients expressed HLA-DR most brightly, we speculate that NK2 bias associated with CD11clow MS would attenuate when NK cells are further activated or differentiated.

NK cell stimulatory proinflammatory cytokines induce up-regulation of CD11c

We next attempted to explore the mechanism(s) for up-regulation of CD11c on NK cells in CD11c high MS. Because both NK cells and CD4+ T cells overexpress HLA-DR in CD11c high, it is probable that immune signals influencing both innate and acquired immunity are operative. So we hypothesized that cytokine signals that have been implicated in the pathogenesis of MS may play a role. We cultured NK cells from HS in the presence or absence of cytokine(s) for 3 days, and evaluated the CD11c expression (MFI). We focused our attention to IL-12, IL-15, and IL-18, which are known to stimulate NK cells with or without help of other cytokines. Notably, they are reportedly elevated in the serum or blood lymphocytes of MS patients as compared with HS (11–14, 18, 19), and prior studies suggest that they may play an important role in autoimmune diseases (20–24). As shown in Fig. 4, although IL-12 and IL-18 showed only a marginal effect on purified NK cells, IL-15 consistently induced 2- to 3-fold up-regulation of CD11c compared with control culture without addition of cytokines. As IL-12 and IL-18 were reported to synergistically work in various settings (25, 26), we then examined whether combinations of these cytokines may induce CD11c. Combination of IL-15 and IL-12 or of IL-15 and IL-18 did not augment the CD11c expression to the level higher than that could be induced by IL-15 alone. However, the combination of IL-12 and IL-18 did up-regulate CD11c on NK cells, which was comparable to the effect of IL-15 alone (Table I).

CD11c high MS relapsed earlier

Given the significant difference in activation status and cytokine phenotype of NK cells as well as HLA-DR expression by CD4+ T cells, it was particularly interesting to know whether CD11c high and CD11c low MS may follow a different clinical course. A new cohort of

**FIGURE 1.** CD11c on NK cells is up-regulated in MS in remission. a, PBMC from HS (n = 10) and MS patients in remission (n = 25) were stained with anti-CD11c-PE, -CD3-ECD, and -CD56-PC5 mAb, and CD11c expression was measured on the CD11c+ fraction gated within whole NK cells (CD11c+/CD3−/CD56+ cells) as mean fluorescence intensity (MFI). Each dot represents the data from individual patients. CD11c high and CD11c low groups of patients are encircled as described in the text. b, In parallel, CD11c expression (MFI) was measured for the whole NK cells (CD3+/CD56+ cells), which yielded a similar result. c, The proportions of CD11c+ cells among whole NK cells are plotted. No significant difference was noted between HS and MS remission. d and e, Representative histogram patterns of CD11c on NK cells (closed histogram) from a single healthy subject (HS) (d) and a patient corresponding to CD11c high MS (e). Open histograms represent isotype control staining. Values represent proportions of CD11c+ fraction (%) and MFI for CD11c+ cells. Mann-Whitney U test was used for statistical analysis. Horizontal bars indicate the mean values. *, p < 0.05; **, p < 0.01.
13 CD11clow and 10 CD11chigh MS patients listed in Table II were followed for up to 120 days. In this preliminary exploration, we set the first episode of relapse after blood sampling as an end point. When the neurologist prescribed corticosteroids without knowing any information on the NK cell phenotype, the patient was considered as the dropout at that time point. Remission rate was calculated as Kaplan-Meier survival rate, and statistical difference between CD11clow and CD11chigh MS was evaluated with the log-rank test (Fig. 5a). At entry, there was no significant difference in the age and disease duration between CD11clow and CD11chigh MS (Table II). On analyzing the collected data after completing the study, we found that 8 patients developed a single relapse during the observation period and that the proportion of patients who have had relapse during the follow-up period was greatly higher in CD11chigh MS (6 of 10, 60%) than in CD11clow MS (2 of 13, 15.3%). Furthermore, the log-rank test revealed that CD11chigh MS relapsed significantly earlier than CD11clow MS (p < 0.003), suggesting a possible role of CD11c as a temporal marker for predicting relapse within months after examination. We also explored whether the difference between CD11chigh and CD11clow could be influenced by age or sex. When we selected a group of patients younger than 38.5 years old (the mean age of all the patients), a significantly earlier relapse in CD11chigh than CD11clow MS was confirmed in this group of patients (p = 0.0067, Fig. 5b). In the rest of the patients (<38.5 years old), the difference was less clear and not significant (p = 0.095). In female patients, CD11chigh MS relapsed significantly earlier than CD11clow MS (p = 0.035, Fig. 5c), whereas this tendency was not statistically significant in male patients (p = 0.083). By examining the patients’ medical records, we also found that the duration from the last relapse tended to be shorter in CD11chigh than CD11clow MS (14.7 ± 12 mo in CD11chigh vs 26.7 ± 24.3 mo in CD11clow) and that the mean number of relapses per year was higher in CD11chigh MS (0.9 ± 0.6 in CD11chigh vs 0.5 ± 0.5 in CD11clow). These are consistent with the postulate that CD11chigh MS might be immunologically more active than CD11clow MS (Table II).

Alteration of CD11c expression in the course of MS

We previously described that NK cells may lose NK2 phenotype during relapse (3). It is interesting to know whether the CD11c phenotype also changes in the course of MS. During the follow-up period of 120 days, 8 patients developed a relapse. We were able to take blood samples at relapse before treatment with corticosteroid and then compared the relapse samples with the samples obtained during remission at initiation of the study. As shown in Fig. 6, we saw an obvious tendency that the levels of CD11c expression would decline during relapse (p < 0.05). HLA-DR expression on NK cells was also reduced in some patients during relapse, but the difference between remission and relapse samples was not statistically significant.

Expression pattern of CD95 vs CD11c on NK cells in MS

In a previous study, we showed that MS patients could be divided into CD95high and CD95low according to the frequency of CD95+ cells among NK cells (4). Additionally, we examined whether expression of CD11c and CD95 may independently reflect the status of MS. We found no significant correlation between CD95 (%) and CD11c (MFI) on NK cells in MS (r = 0.29, p = 0.16 with Spearman’s correlation coefficient by rank test), indicating that expression of CD95 and CD11c on NK cells may be regulated independently. By setting the upper limits of CD95+ (%) and CD11c MFI as (the average + 2 × SD) of HS (CD95: 44.6%, CD11c: 5.04),
we then examined whether there is a correlation between CD11c
CD95 phenotype and clinical conditions (Fig. 7). Naturally, all the
healthy subjects were plotted in the left lower quadrant
(CD95lowCD11clow). In contrast, MS patients were plotted in all
the four quadrants with differential proportions of patients who
have no relapse during 120 days: CD95lowCD11clow; 3/3 (100%),
CD95lowCD11chigh; 1/2 (50%), CD95highCD11clow; 8/10 (80%),
CD95highCD11chigh; 2/7 (28.6%). Although the data for CD95low
subjects (lower left and lower right) need to be omitted due to the
limited sample size, we found that the difference between
CD95highCD11clow and CD95highCD11chigh in remission rate was
significant with log-rank test (p = 0.028). Provided that CD95high
patients possessed an increased frequency of memory autoreactive
T cells (4), this result is consistent with the idea that when com-
parable numbers of autoimmune T cells are present in the periph-
eral circulation, remission of MS is more stable in patients with
CD11clow NK cells.

Discussion
Blood examination of systemic autoimmune diseases such as sys-
temic lupus erythematosus usually exhibits measurable abnormalities
such as elevation of autoantibodies, which is useful for eval-
uating activity of disease. In contrast, patients with MS do not
accompany such systemic abnormalities in laboratory tests except

FIGURE 3. IL-5 and GATA-3 mRNA are in-
creased in CD11clow but not in CD11chigh MS. Total
RNAs were extracted from purified NK cells of HS
(n = 8), CD11clow (n = 9), or CD11chigh MS (n = 8). mRNA expression of IL-5 (a), GATA-3 (b),
IFN-γ (c), and T-bet (d) was evaluated by quantita-
tive PCR. The data are normalized to endogenous
ß-actin expressions in the same samples. ANOVA
was used for statistical analysis. Horizontal bars in-
dicate the mean values, *, p < 0.05; **, p < 0.01.

FIGURE 4. CD11c expression on NK cells is up-
regulated with addition of IL-15. a, Purified NK cells
were cultured in the absence or presence of IL-12,
IL-18, or IL-15. Three days later, the cells were
stained with anti-CD11c-PE, -CD3-ECD, and
-CD56-PC5 mAb. CD11c expression on NK cells
(CD3−CD56− cells) is demonstrated as single histo-
gram. Values indicate CD11c MFI of CD11c− frac-
tions. A representative of three independent experi-
ments is shown. b, Data are expressed as mean fold
increase of CD11c MFI (the MFI in the presence of
cytokine/the MFI in the absence of cytokine) + SD
from three independent experiments. ANOVA was
used for statistical analysis. **, p < 0.01.
in unusual cases. It is currently recognized that autoreactive T cells might be activated and expanded to various degrees in the peripheral blood and peripheral lymphoid organs of MS even during remission (1–4). In fact, our previous work suggests that a higher number of memory autoreactive T cells is linked with unstable disease course (4). If we are able to accurately evaluate the immune status of each patient with a relatively simple test, it should be most helpful in treatment and management of MS. In this line, it is currently of particular importance to identify measurable indicators which would serve as clinically appropriate biomarkers in MS (2).

This study has clarified for the first time to our knowledge that CD11c expression on peripheral NK cells is significantly up-regulated in a major proportion of patients with MS in remission. To obtain insights into the mechanism and the biological meaning of the NK cell expression of CD11c in autoimmune disease MS, we have attempted to clarify the difference between CD11c<sup>high</sup> and CD11c<sup>low</sup> patients regarding phenotypes of NK cells, cytokine profile, and temporal clinical activity. We also explored which inflammatory cytokines might induce CD11c on NK cells. According to the NK cell expression of CD11c, we have classified the patients with MS in remission into CD11c<sup>high</sup> and CD11c<sup>low</sup>. Most notably, NK2 phenotype characterized by predominant IL-5 production was seen in CD11c<sup>low</sup> patients, but not in CD11c<sup>high</sup>. Consistently, the CD11c<sup>high</sup> patients were found to be clinically more active than CD11c<sup>low</sup> as judged by the remission rate during the 120 days after examination. These results indicate that up-regulation of CD11c on NK cells would reflect the temporal disease activity and therefore could be used to identify patients who are likely to exacerbate within months. It has been reported that CD11c<sup>+</sup> NK cells in mice could serve as APCs (6, 7). However, we could not reveal Ag presenting capacity of human CD11c<sup>+</sup> NK cells (data not shown).

Regarding the mechanism of CD11c induction on NK cells, we have found that in CD11c<sup>high</sup> patients, HLA-DR is concomitantly up-regulated with CD11c on NK cells (Fig. 2), which suggests that up-regulation of CD11c on NK cells would reflect the temporal disease activity and therefore could be used to identify patients who are likely to exacerbate within months. It has been reported that CD11c<sup>+</sup> NK cells in mice could serve as APCs (6, 7). However, we could not reveal Ag presenting capacity of human CD11c<sup>+</sup> NK cells (data not shown).

| Table I. Effect of several cytokines on CD11c expression on NK cells |
|------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| No Cytokine         | IL-12 | IL-18 | IL-15 | IL-12 + IL-18 | IL-4 | TNF | GM-CSF | IL-23 | IL-8 |
| Expt. 1             | 1.00* | 1.19 | 1.57 | 2.90 | ND | ND | ND | ND | ND |
| Expt. 2             | 1.00 | 1.04 | 1.43 | 2.96 | 2.86 | ND | ND | ND | ND |
| Expt. 3             | 1.00 | 1.59 | 1.25 | 2.53 | 3.44 | ND | ND | ND | ND |
| Expt. 4             | 1.00 | ND | ND | 2.62 | ND | 1.19 | 1.10 | 0.95 | 1.14 |
| Expt. 5             | 1.00 | ND | ND | 2.81 | ND | 1.24 | 1.10 | 1.05 | 1.05 |
| Mean               | 1.00 | 1.27 | 1.42 | 2.77 | 3.15 | 1.21 | 1.10 | 1.00 | 1.00 |
| SD                 | 0.00 | 0.29 | 0.16 | 0.19 | 0.41 | 0.03 | 0.07 | 0.07 | 0.07 |

* Purified NK cells were stimulated with cytokines. Data are expressed as fold increase of CD11c MFI (the MFI in the presence of the indicated cytokines/the MFI in the absence of cytokines) in the presence of indicated cytokines. More than a 2-fold increase is highlighted (bold).

| Table II. Information on the patients whose clinical courses were followed for up to 120 days |
|------------------------|------------------|------------------|------------------|
| Identification No. | Group | Age (years) | Sex | Disease Period (Years) | Total Number of Relapses | Duration from the Last Relapse (mo) | Mean Numbers of Relapse/Year |
| 1                  | Low   | 17        | F*  | 9.6              | 2                  | 24                   | 0.2                          |
| 2                  | Low   | 52        | M   | 12.2             | 9                  | 3                    | 0.7                          |
| 3                  | Low   | 31        | F   | 6.2              | 13                 | 7                    | 2.1                          |
| 4                  | Low   | 32        | F   | 3.9              | 1                  | 34                   | 0.3                          |
| 5                  | Low   | 42        | F   | 2.2              | 1                  | 8                    | 0.5                          |
| 6                  | Low   | 35        | M   | 20               | 3                  | 88                   | 0.2                          |
| 7                  | Low   | 37        | M   | 8.5              | 3                  | 50                   | 0.4                          |
| 8                  | Low   | 35        | F   | 2.4              | 1                  | 38                   | 0.5                          |
| 9                  | Low   | 26        | F   | 4.8              | 2                  | 10                   | 0.4                          |
| 10                 | Low   | 26        | F   | 1.5              | 1                  | 8                    | 0.7                          |
| 11                 | Low   | 41        | M   | 5.5              | 1                  | 24                   | 0.2                          |
| 12                 | Low   | 64        | F   | 4.5              | 2                  | 8                    | 0.4                          |
| 13                 | Low   | 42        | F   | 6.3              | 1                  | 45                   | 0.2                          |
| Mean + SD          |      | 36.9 ± 12.0 | 6.7 ± 5.0 | 3.1 ± 3.7 | 26.7 ± 24.3 | 0.5 ± 0.5 |
| 14                 | High  | 39        | M   | 4.4              | 2                  | 22                   | 0.5                          |
| 15                 | High  | 31        | F   | 9.2              | 11                 | 14                   | 1.2                          |
| 16                 | High  | 46        | F   | 7.4              | >20<sup>b</sup>    | 2                    | ND                           |
| 17                 | High  | 53        | F   | 2.1              | 4                  | 5                    | 1.9                          |
| 18                 | High  | 59        | F   | 4.9              | 2                  | 19                   | 0.4                          |
| 19                 | High  | 27        | M   | 9.3              | 4                  | 9                    | 0.4                          |
| 20                 | High  | 36        | F   | 2.7              | 1                  | 19                   | 0.4                          |
| 21                 | High  | 34        | F   | 3.8              | 2                  | 43                   | 0.5                          |
| 22                 | High  | 60        | F   | 3.4              | 6                  | 10                   | 1.8                          |
| 23                 | High  | 21        | F   | 1.8              | 2                  | 4                    | 1.1                          |

* F, Female; M, male.

<sup>b</sup> This value is eliminated from calculation of the mean.
vitro CD11c induction on NK cells may recapitulate the phenotypic alteration of NK cells in CD11c<sup>high</sup> patients. Interestingly, IL-18 is not only a cytokine able to facilitate IFN-γ production by NK cells in cooperation with IL-12 (25, 26) but is crucial in inducing pathogenic autoimmune responses (21). Furthermore, autoimmune encephalitogenic T cells can induce more serious disease upon adoptive transfer when they are preactivated in the presence of IL-12 and IL-18 (20). Taken together, these results allow us to speculate that the proinflammatory cytokines may be involved in the up-regulation of CD11c on NK cells. Although the relationship between serum cytokine concentration and levels of CD11c expression on NK cells should be estimated in future studies, a previous work (11, 29, 30) showing that a probable link between IL-15 and temporal disease activity, indicates that NK cell expression of CD11c is likely to correlate with the levels of cytokines.

In the Th cell differentiation, specific transcription factors have been identified that play a crucial role in inducing Th1 or Th2 cells. Namely, Th1 differentiation characterized by IFN-γ induction requires a transcription factor T-bet, whereas GATA-3 and c-maf act to promote Th2 cytokine production (31–33). Human NK cells cultured in the presence of IL-12 or IL-4 differentiate into NK1 or NK2 populations, reminiscent of Th1 and Th2 cells (5). Whereas NK1 cells produce IL-10 and IFN-γ, NK2 cells would serve as immune regulators by producing IL-5 and IL-13. Notably, up-regulation of GATA-3 has been reported in mouse NK2 cells (17), raising a possibility that Th cells and NK cells might share the same transcription factor for inducing the key cytokines. We have previously reported that IL-5 expression is one of the characteristics of NK cells in the remission state of MS (3). However, it was not excluded that overexpression of IL-5 could be restricted to a proportion of patients. Here, we have addressed whether NK cells from CD11c<sup>high</sup> and CD11c<sup>low</sup> MS may differ with regard to expression levels of IL-5 and GATA-3. By measuring the mRNAs, we found that expression levels of IL-5 and GATA-3 are elevated in CD11c<sup>low</sup> MS but not in CD11c<sup>high</sup> (Fig. 3). Furthermore, we showed that...
indicates that CD95highCD11chigh MS may be most unstable sub-
activity as well as functional alteration of regulatory NK cells. Our
judged as being in clinical remission.

Among the patients whose clinical state could be
tended to be shorter and the mean number of relapses per year
relapsed during the 120 days follow-up period.

FIGURE 7. Expression pattern of CD95 vs CD11c on NK cells from
MS. PBMC from MS or HS were stained with CD95-FITC, CD11c-PE,
CD3-ECD, and CD56-PC5. After determining the proportion of CD95+

neither IFN-γ nor T-bet was increased in CD11chigh MS. This
suggests that NK cells from CD11clow are NK2-biased but those
from CD11chigh are not, although MS in remission as a whole is
NK2-biased as compared with control subjects. More recently, we
have observed that stimulation with IL-15 or IL-12 plus IL-18
would decrease IL-5 and GATA-3 mRNA in purified NK cells
with reciprocal up-regulation of CD11c (data not shown). This
further supports a model that proinflammatory cytokines may play
a crucial role in the absence of NK2 bias in CD11chigh MS.

To clarify the clinical differences between CD11chigh and
CD11clow, we followed up the clinical course of the patients after
blood sampling. Although there was no significant difference in
clinical parameters at examination of NK cells, we have found that
CD11chigh MS showed a significantly earlier relapse than
CD11clow MS. This is consistent with our assumption that the
absence of NK2 bias in CD11chigh MS should imply that regulatory
NK cell functions are defective in this group of patients. When we
reanalyzed the data regarding various clinical parameters, we
found that an earlier relapse in CD11chigh than CD11clow MS is
more remarkable in the younger group (<38.5 years old) or in
female patients. Furthermore, the duration from the last relapse
tended to be shorter and the mean number of relapses per year
higher in CD11chigh MS, supporting that CD11chigh MS is more
active than CD11clow MS.

When we analyzed expression of CD95 and CD11c on NK cells
simultaneously, we found that MS patients in remission could be
divided into four subgroups (Fig. 7). When we compared clinical
course after examination of NK cell phenotypes, we found that
CD95highCD11chigh MS relapsed significantly earlier than
CD95highCD11clow MS (p = 0.028 with log-rank test). This result
indicates that CD95highCD11chigh MS may be more unstable
subgroup of MS, among the patients whose clinical state could be
judged as being in clinical remission.

In this study, we have demonstrated that MS patients
differentially express CD11c on peripheral blood NK cells and a higher
expression of CD11c on NK cells may reflect the temporal disease
activity as well as functional alteration of regulatory NK cells. Our
results have a clinical implication because of a lack of appropriate
biomarker to monitor the immunological status in MS at present.

To verify the reliability of this marker, longitudinal examination of
CD11c expression on NK cells in the same patients should be
performed in the future study.

Disclosures
The authors have no financial conflict of interest.

References
3. Takahashi, K., S. Miyake, T. Kondo, K. Terao, M. Hatakenaka, S. Hashimoto, and
5. Perret, S., S. Roberts, T. Bao, K. P. Erhard, B. Coon, J. M. E. Wulks, and
J. J. P. Neijenhuis. 1998. Differentiation of human NK cells into NK1 and NK2 subsets.
6. Homann, D., A. Jahreis, T. Wolfle, A. Hughes, B. Coon, M. J. van Stipdonk,
K. R. Prilliman, S. P. Schoenberger, and M. G. von Herrath. 2002. CD40L block-
ade prevents autoimmune diabetes by induction of bitypic NK/DC regulatory cells.
Natural killer dendritic cells have both antigen presenting and lytic function and in
p150,95 (CD11c/CD18) as a receptor for iC3b: activation by a heterologous β subunit
and localization of a ligand recognition site to the I domain. J. Immunol. 152:
4582–4589.
functions as an adhesion molecule binding to a counter-receptor on stim-
2003. Internalization of circulating apoptotic cells by splenic marginal zone den-
dritic cells: dependence on complement receptors and effect on cytokine produc-
2002. Increased levels of IL-15 mRNA in relapsing-
and interleukin-18 in progressive multiple sclerosis: induction
Increased interleukin 12 production in progressive multiple sclerosis: induction
by activated CD4+ T cells via CD40 ligand. Proc. Natl. Acad. Sci. USA 94:
599–603.
2002. IL-18 is linked to raised IFN-γ in multiple sclerosis and is induced by
activated CD4+ T cells via CD40-CD40 ligand interactions. J. Neuroimmunol. 125:
134–140.
15. McDonald, W. I., A. Compton, G. Edan, D. Gokdin, H. P. Hartung, F. D. Lublin,
Recommended diagnostic criteria for multiple sclerosis: guidelines from the
16. Lima, M., J. Almeida, M. dos Anjos Teixeira, M. L. Queiros, B. Justina, and
CD11b, CD45RA/CD45RO, and CD11a/HLA-DR expression identify acute/early
17. Katsumoto, T., M. Kimura, M. Yamaishi, H. Hosokawa, K. Hashimoto, A. Hasegawa,
K. Bendzhen, and A. Reggio. 2001. Increased serum levels of interleukin-18 in
19. Ito, A., A. Matejuk, C. Hopke, H. Drought, J. Dwyer, A. Zamora,
S. Subramanian, A. A. Vandenbark, and H. Offner. 2003. Transfer of severe
experimental autoimmune encephalomyelitis by IL-12- and IL-18-potenti-
directs autoreactive T cells and promotes autodestruction in the central nervous
M. Nakanishi, G. N. Takeda, T. Akira, and G. Trinchieri. 1999. NK cell activity and


