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Natural killer cell phenotype and clinical response to interferon-beta therapy in multiple sclerosis

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Abstract CD56^{bright} NK cells, which may play a role in immunoregulation, are expanded in multiple sclerosis (MS) patients treated with immunomodulatory therapies such as daclizumab and interferon-beta (IFNβ). Yet, whether this NK cell subset is directly involved in the therapeutic effect is unknown. As NK receptor (NKR) expression by subsets of NK cells and CD8+ T lymphocytes is related to MS clinical course, we addressed whether CD56^{bright} NK cells and NKR in IFNβ-treated MS patients differ according to the clinical response. IFNβ was associated to lower LILRB1+ and KIR+NK cells, and higher NKG2A+NK cell proportions, an immunophenotypic pattern mainly found in responders. After IFNβ treatment, a CD56^{bright} NK cell expansion was significantly related to a positive clinical response. Our results reveal that IFNβ may promote in responders changes in the NK cell immunophenotype, corresponding to the profile found at early maturation stages of this lymphocyte lineage.

1. Introduction

NK cells are lymphocytes involved in the innate immune defense against infections and tumors. NK cells may as well display additional functions related with immunoregulation of adaptive immune responses [1–3]. In contrast to T and B lymphocytes, NK cells lack antigen-specific receptors but instead express a variegated repertoire of activating and inhibitory NK cell receptors (NKR). The balance between their opposite signals regulates NK cell activation. Some NKR are widely expressed by most NK cells whereas others (e.g. KIRs, NKG2, LILRB1) are selectively expressed by NK and T cell subsets [4–7].

Several studies have implicated NK cells in multiple sclerosis (MS). A reduction in the number and function of NK

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cells may precede the development of relapses and new brain lesions [8–10]. NKR expression by NK cells and T CD8+ lymphocytes may differ in the peripheral blood of MS patients according to age and clinical course of the disease, presumably stimulated by a persistent challenge, which may be modulated by treatment with interferon-beta (IFNβ) [11]. Some studies have described an expansion of the CD56^{bright} NK cell subset in patients treated with immunomodulatory therapies such as daclizumab and IFNβ [9,12]. CD56^{bright} NK cells may play an immunoregulatory function over adaptive T cell responses [9] and their NKR expression pattern differs from that displayed by CD56^{dim} NK cells, presumably reflecting different stages of NK cell differentiation [13–15]. Remarkably, the expansion of CD56^{bright} NK cells after daclizumab treatment has been correlated with a positive clinical and radiological response in MS patients; yet, whether this NK cell subset is directly involved in the therapeutic process is unknown. The mechanism(s) leading to CD56^{bright} NK cell expansion by these immunomodulatory therapies are partially understood [16] and may be unrelated with the specific MS pathological background, since an analogous expansion may be observed in other diseases treated with similar drugs (e.g., interferon-alpha in chronic hepatitis C, daclizumab in uveitis, rituximab in rheumatoid arthritis) [17–19].

In the present study, we addressed whether expansion of CD56^{bright} NK cells and NKR expression by NK and CD8+ T cells were associated with the clinical response to IFNβ therapy in MS patients. Baseline evaluation of NKR expression did not allow the prediction of clinical response to IFNβ; nevertheless, this therapy was related with an expansion of CD56^{bright} NK cells mainly in clinical responders, who displayed additional changes in NKR expression. These results reveal novel biological pathways potentially related with the therapeutic effect of this immunomodulatory therapy in MS.

2. Material and Methods

2.1. Patients

A case–control cohort of patients were evaluated at the outpatient MS clinic of Hospital del Mar, Barcelona; they included 27 MS patients treated with IFNβ for at least 6 months and 17 untreated MS with no further clinical differences. In order to evaluate clinical response to IFNβ, a historical prospective cohort of patients were studied at the outpatient MS clinic of the Centre d'Esclerosi Múltiple de Catalunya (CEM-Cat), Barcelona; this cohort was formed by 44 patients evaluated at baseline prior to the beginning of IFNβ therapy; 25 patients were also evaluated after 2 years of IFNβ treatment. None of the patients have received treatment with corticosteroids within 1 month prior to the baseline or follow-up analysis. Forty-one healthy controls of similar age and gender distribution were included in the study. All patients were older than 18 years and fulfilled McDonald criteria of MS at the time of inclusion. The study was approved by the local ethics committees, and all patients gave their informed consent. Clinical variables analyzed were age and disease duration, sex, the Expanded Disability Status Scale (EDSS), annualized relapse rate and number of relapses in the previous 2 years, MS form (relapsing–remitting (RRMS), secondary progressive MS (SPMS)) and type of IFNβ treatment after the baseline analysis. In a subgroup of MS patients, information on brain MRI scans such as number of gadolinium (Gd)-enhancing lesions and T2 lesion load [20] was also available at baseline (n = 22) and after 12 months of IFNβ treatment (n = 15). Clinical criteria of response to IFNβ were applied after 2 years of treatment and defined as previously reported [21]; briefly, patients were classified as responders if there was no increase in the EDSS and no relapses during the follow-up period. Non-responders were defined when patients experienced one or more relapses and an increase in the EDSS of at least one point in two consecutive visits separated by a 6-month period. Patients with intermediate phenotypes of response (i.e., presence of relapses and increase in the EDSS < 1.0 or absence of relapses with higher increases in the EDSS) were not included in the study in order to clearly discriminate between both groups.

2.2. Immunofluorescence and Flow Cytometry Analysis

Heparinized peripheral blood was obtained by venous puncture and peripheral blood mononuclear cells (PBMC) were isolated using a Ficoll-Hypaque density gradient centrifugation and cryopreserved until evaluation. An indirect immunofluorescence staining was performed in the Immunology Unit, Universitat Pompeu Fabra, Barcelona, Spain, pretreating cells with saturating concentrations of human aggregated Ig to block FcγR and subsequently labeled at saturating concentrations with different monoclonal antibodies (mAbs). HP-F1 anti-LILRB1 was generated in our laboratory as previously reported [22]. Z199 anti-CD94/NKG2A and BAT221 anti-NKG2D mAbs were provided by Dr. A. Moretta (University of Genova, Italy). MAB1381 anti-NKG2C was from R&D systems (Minneapolis, MN). As previously described [23], KIR staining was performed using a mixture of mAbs specific for: KIR3DL1/L2/S4 (5.133), provided by Dr. M. Colonna; KIR2DL2/S2/L3 (CH-L), provided by Dr. S. Ferrini (University of Genova, Italy); KIR3DL1 (Dx9), provided by Dr. L. Lanier (UCSF, San Francisco, CA); and KIR2DL1/S1/S3 (HP-3E4), generated in our laboratory. Control experiments were carried out to verify that the cryopreservation process did not alter the expression of these NKR. Four-color staining was performed incubating the individual primary antibodies followed by washing and labeling with phycoerythrin PE-tagged F $(ab')_2$ rabbit anti-mouse Ig antibodies (Dako, Glostrup, Denmark); subsequently, samples were incubated with anti-CD3-peridin chlorophyll protein (PerCP) and anti-CD56-allophycocyanin (APC) (BD Bioscience Pharmingen), and anti-CD8-fluorescein isothiocyanate (FITC) (Immunotools, Friesoythe, Germany). NK cells were defined as CD3-CD56+ lymphocytes; CD56^{dim} and CD56^{bright} NK cell subsets were identified according to the staining intensity with the specific mAb. CD8+ T cells were defined as CD3+CD8+ lymphocytes, selecting CD8^{bright} cells for NKR analysis. Flow cytometry data were expressed as the percentages of specifically stained cells and as an increase in percentage relative to baseline levels. Absolute numbers of NK cells were calculated when blood counts obtained in parallel were available (all MS

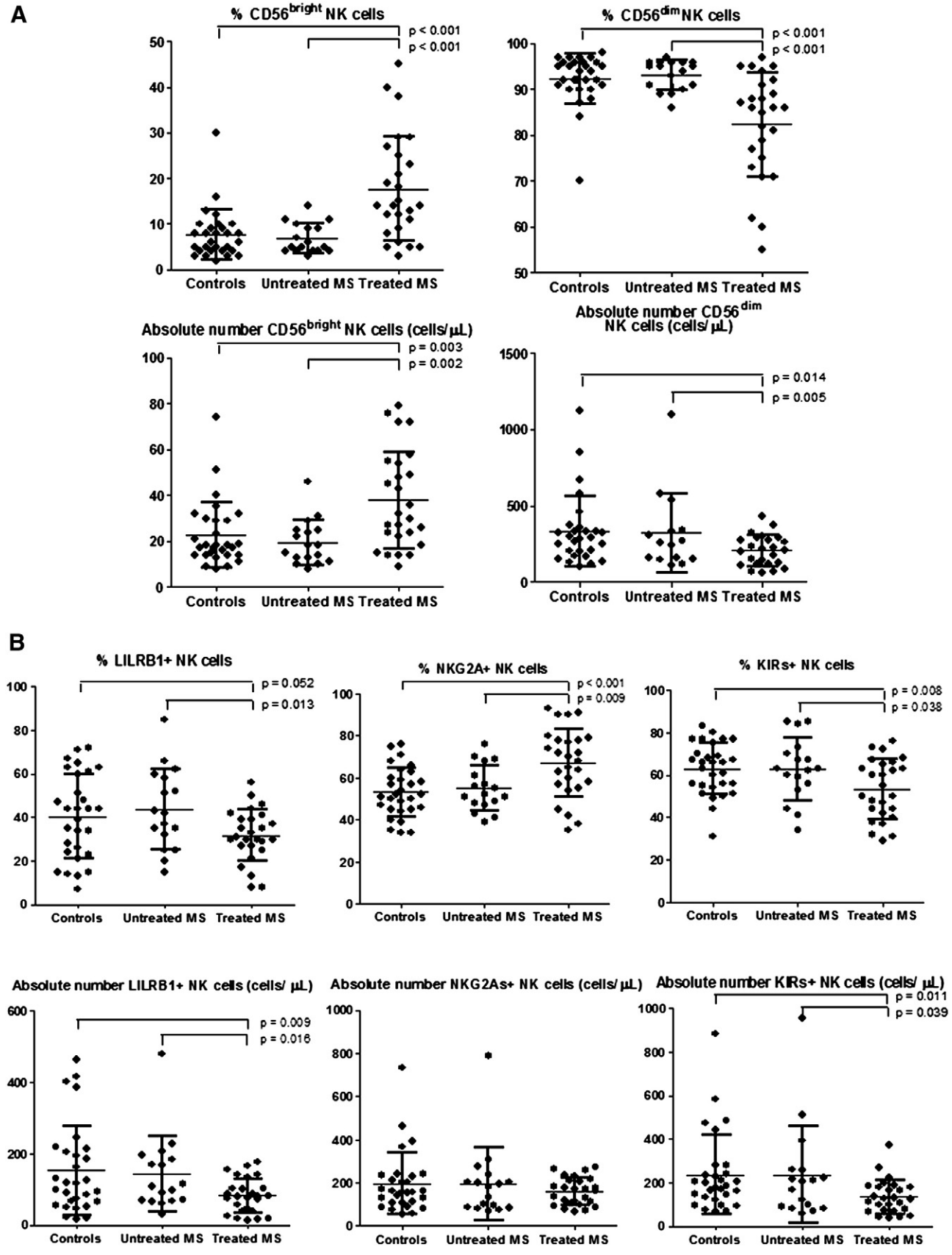

Figure 1 NK cell subsets and NKR expression according to IFNβ-therapy. CD56^{bright} and CD56^{dim} NK cells (A), and NKR expression (B) (LILRB1, NKG2A, KIRs) in controls, untreated MS and IFNβ-treated MS patients. Data are expressed as percentage of NK cells and absolute numbers of NK cells in peripheral blood. p Value: Student's *t* test.

patients from the case–control study and 29 healthy controls). The immunological evaluation was performed by a single investigator (JEMR) without knowledge of the clinical data.

2.3. Statistical Analysis

Normal Q–Q probability plots assessed normal distribution of continuous variables, and depending on the applicability conditions, Student's *t*-test and Mann–Whitney *U* test were used to analyze differences between independent variables. Differences in NKR expression and percentage of CD56^{bright} NK cells between the baseline period and after 2 years of IFNβ treatment were evaluated by the Wilcoxon signed rank test. Pearson and Spearman correlations were calculated for pair-wise continuous variables. Results were considered significant at the two-sided level of 0.05, using Bonferroni correction to adjust significance level for multiple comparisons.

3. Results

3.1. NK Cell Subsets in IFNβ-treated MS Patients

The effect of IFNβ therapy on NK cell populations was studied in a case–control cohort of patients. IFNβ-treated MS patients (n=27) as compared to untreated patients (n=17) showed higher proportions and absolute numbers of CD56^{bright} NK cells, but lower CD56^{dim} NK cells. NKR expression by NK cells also differed according to IFNβ therapy; in this regard, IFNβ-treated MS patients displayed higher NKG2A but lower LILRB1 and KIR expression by NK cells (Fig. 1). Moreover, NKR expression and the relative numbers of CD56^{bright} and CD56^{dim} NK cells in untreated MS patients were comparable with healthy controls (n=29) (Fig. 1). No differences were found when the percentage or absolute numbers of NK cells were analyzed, except for NKG2A expression (Fig. 1).

Table 1 Baseline characteristics and NKR expression of MS patients prior to IFNβ treatment. [a] MS patients are subdivided according to the clinical response to IFNβ defined after 2 years of treatment.

Characteristics	MS patients (n=44)	Controls (n=41)	p-Value	IFNβ responders (n=22)	IFNβ non-responders (n=22)	p-Value
Age (years)	36.4 (8.8)	38.1 (11.0)	0.435	35.3 (8.4)	37.5 (9.4)	0.510
MS duration (years)	4.6 (4.9)			3.7 (4.6)	5.6 (5.2)	0.066
Female/male (ratio)	30/14 (2.1)	28/13 (2.2)	0.588	14/8 (1.75)	16/6 (2.7)	0.517
MS form (RRMS/SPMS) (% SPMS)	37/7 (16%)			21/1 (4.5%)	16/6 (27%)	**0.039**
EDSS	2.5 (1.3)			2.1 (1.0)	3.0 (1.4)	**0.042**
Annualized relapse rate [b]	1.9 (1.0)			1.9 (1.0)	1.9 (1.1)	0.854
Number of Gd-enhancing lesions [c]	1.7 (3.3)			1.9 (3.7)	1 (2)	0.590
T2 lesion load [c]	73.1 (69.9)			72.2 (77.2)	75.3 (51.3)	0.747
Type of IFNβ:						
sc 1a				8 (36.5%)	5 (23%)	0.294
sc 1b				6 (27%)	11 (50%)	
im 1a				8 (36.5%)	6 (27%)	
CD8+ T cells						
Percentage	23.5 (6.5)	21.6 (6.7)	0.197	22.4 (7.4)	24.6 (5.4)	0.169
NKG2D	96.7 (2.3)	96.1 (4.9)	0.517	96.5 (2.3)	96.8 (2.2)	0.673
LILRB1	27.4 (15.9)	25.3 (16.9)	0.563	29 (15.2)	25.9 (16.8)	0.481
NKG2A†	3.0 (3.0–5.75)	4.0 (2.0–6.0)	0.724	3.5 (3.0–5.25)	3.0 (2.0–6.0)	0.542
NKG2C†	3.0 (1.25–5.0)	2.0 (1.0–3.0)	**0.013**	3.0 (1.75–5.0)	3.0 (1.0–6.0)	0.868
KIR	10.6 (6.2)	8.7 (6.9)	0.191	10.6 (4.4)	10.7 (7.8)	0.316
NK cells						
Percentage	12.1 (7.3)	18.4 (8.6)	<**0.001** [d]	12.8 (8.8)	11.4 (5.5)	0.787
NKG2D	97.4 (2.1)	97.3 (2.7)	0.831	97.4 (2.2)	97.4 (2)	0.961
LILRB1	45.4 (16.7)	39.8 (18)	0.136	48.8 (18)	42.1 (15)	0.155
NKG2A	49.4 (14.9)	53 (12.5)	0.225	47.4 (14.8)	51.3 (15)	0.518
NKG2C	21.1 (13.1)	17.6 (14.1)	0.302	21.3 (14.2)	20.9 (12.5)	0.963
KIR	65.5 (11.5)	60.1 (12.6)	0.053	68.1 (10.1)	62.7 (12.4)	0.136
CD56^{bright}	8 (5.3)	7.1 (4.9)	0.417	7.7 (5.9)	8.3 (4.8)	0.340

p-value: Mann–Whitney *U* test. A p-value < 0.05 is bold emphasized.

[a] NKR expression value are expressed as mean % (SD), except for non-parametric variables marked with † that were expressed as median (q1–q3).

[b] Refers to the number of relapses in the two previous years.

[c] MRI data was available for 22 MS patients (16 responders, 6 non-responders).

[d] Significant p-value after Bonferroni correction.

Table 2 MS patients and NKR expression after 2 years of IFNβ treatment according to the clinical response. [a]

Characteristics	MS patients (n=25)			Non-responders (n=7)			Responders (n=18)		
	Baseline	IFNβ	p-Value	Baseline	IFNβ	p-Value	Baseline	IFNβ	p-Value
EDSS	2.5 (1.3)	2.6 (1.8)	0.733	3.4 (1.5)	4.7 (1.3)	**0.042**	2.1 (1.0)	1.8 (1.2)	0.062
Annualized relapse rate [b]	1.9 (1.0)	0.6 (1.2)	**0.002** [d]	2.0 (1.2)	2.3 (1.1)	0.832	1.9 (1.0)	0	<**0.001** [d]
Number of Gd-enhancing lesions [c]		0.40 (0.83)	**0.020**						
T2 lesion load [c]		63.5 (50.1)	0.413						
Type of IFN-b:									
sc 1a		7 (28%)			1 (14%)			6 (33%)	0.236
sc 1b		8 (32%)			4 (57%)			4 (22%)	
im 1a		10 (40%)			2 (29%)			8 (45%)	
CD8+ T cells									
Percentage	22.4 (7.0)	21.0 (7.5)	0.113	23.1 (4.6)	19.4 (6.2)	0.051	22.1 (7.8)	21.7 (8)	0.659
NKG2D	96.2 (2.6)	95.6 (3.2)	0.233	96.0 (3.1)	94.0 (4.5)	0.090	96.2 (2.5)	96.2 (2.3)	0.905
LILRB1	30.3 (14.9)	27.4 (15.7)	0.332	35.0 (11.9)	26.6 (14.9)	0.236	28.5 (15.8)	27.7 (16.4)	0.827
NKG2A†	4.0 (3.0–6.0)	4.0 (3.0–7.0)	0.127	5.1 (2.5)	5.1 (4.1)	1.0	3.5 (3.0–5.25)	4.0 (3.0–6.5)	0.080
NKG2C†	3.0 (1.5–7.0)	3.0 (1.0–5.0)	0.303	8.4 (7.8)	4.1 (4.3)	0.058	2.5 (1.0–5.0)	3.0 (1.75–5.0)	0.681
KIR	11.7 (6.2)	10.0 (7.3)	0.106	15.2 (9.5)	10.1 (6.5)	0.104	10.6 (4.5)	9.9 (7.8)	0.49
NK cells									
Percentage	12.0 (6.3)	12.8 (5.7)	0.425	12.9 (7.6)	15.4 (6)	0.223	11.6 (5.9)	11.7 (5.3)	0.849
NKG2D	97.6 (2.1)	97.2 (2.5)	0.191	97.7 (1.6)	97.6 (1.9)	0.773	97.6 (2.3)	97.1 (2.7)	0.244
LILRB1	46.0 (16.8)	39.0 (13.3)	**0.0007** [d]	40.6 (12.9)	34.6 (9.5)	**0.042**	48.2 (17.9)	40.9 (14.5)	**0.006** [d]
NKG2A	48.4 (14.0)	55.5 (15.4)	<**0.0001** [d]	51.4 (11.4)	57 (12.9)	0.108	47.3 (15.1)	54.9 (16.6)	**0.0002** [d]
NKG2C	20.4 (13.7)	22.6 (15.1)	0.063	17.7 (10.1)	17.5 (10.5)	0.809	21.8 (15.4)	25.7 (17.1)	0.525
KIR	66.0 (10.0)	62.6 (9.8)	0.082	60 (10.7)	59.7 (8.5)	0.865	68.4 (8.9)	64.1 (10.3)	**0.048**
$CD56^{bright}$	7.7 (5.4)	12.0 (6.7)	<**0.0001** [d]	8.9 (3.2)	11.3 (2.8)	0.058	7.3 (6.1)	12.3 (7.7)	**0.0007** [d]

p-value: Wilcoxon signed rank test. A p-value < 0.05 is bold emphasized.

[a] NKR expression value are expressed as mean % (SD), except for non-parametric variables marked with † that were expressed as median (q1–q3).

[b] Refers to the number of relapses in the two previous years.

[c] MRI data were available for 22 MS patients (16 responders, 6 non-responders).

[d] Significant p-value after Bonferroni correction.

3.2. CD56^{bright} NK Cells and NKR Expression at Baseline

We evaluated 44 MS patients prior to the initiation of IFNβ therapy in a historic prospective cohort. Clinical characteristics and NKR expression by CD8+ T lymphocytes and NK cells in MS patients and controls are shown in Table 1. Demographic and baseline clinical and radiological characteristics were comparable between responders (n=22) and non-responders (n=22) to IFNβ, except for a higher EDSS in non-responders, a pattern already described in previous studies [24], and a higher number of SPMS forms in the group of non-responders.

The percentage of CD56^{bright} NK cells and NKR expression were comparable between MS patients and controls, except for lower NK cell proportions in MS patients ($p<0.001$). An additional analysis of NKR expression in the CD56^{bright} NK cell subset did not show differences in MS patients compared with controls (data not shown). No significant correlations were observed between radiological variables and CD56^{bright} NK cells or NKR expression at baseline (data not shown).

As previously reported [11], LILRB1 expression by CD8+ T cells in MS patients was directly related with age ($r=0.48$, $p<0.01$). A subsequent analysis according to the clinical response to IFNβ showed that the age-related LILRB1 expression by CD8+ T cells was mainly found in non-responders ($r=0.58$, $p=0.005$) as compared to responders ($r=0.35$, $p=0.11$) (Supplementary Fig. 1). No further immunophenotypic differences were noted between responders and non-responders at baseline (Table 1).

3.3. CD56^{bright} NK Cells and NKR Expression after IFNβ Treatment

In a subgroup of 25 patients (18 responders and 7 non-responders) studied after 2 years of treatment with IFNβ changes in NKR expression and expansion of CD56^{bright} NK cells were evaluated. As shown in Table 2, treatment with IFNβ was associated with a significant expansion of CD56^{bright} NK cells (mean values ranging from 7.7% (SD 5.4) at baseline to 12.0% (6.7) after 2 years of treatment; $p<0.0001$); as shown in Fig. 2, this effect was observed with all types of IFNβ.

In addition to the expansion of CD56^{bright} NK cells, the expression of NKR by NK cells was also significantly modified by IFNβ therapy (Table 2). The percentage of LILRB1+ NK cells decreased ($p=0.0007$) and NKG2A+NK cells increased ($p<0.0001$) after IFNβ therapy. These differences were still maintained when the analysis was focused on the subgroup of CD56^{dim} NK cells, thus excluding any possible bias in the results secondary to the expansion of CD56^{bright} NK cells (e.g., a subset with lower and higher expression of LILRB1 and NKG2A, respectively) [25]. In a subgroup of 15 patients evaluated by MRI 1 year after IFNβ, the number of gadolinium-enhanced lesions was reduced as compared to the baseline period after IFNβ treatment (1.4 (2.1) vs. 0.4 (0.8), $p=0.02$). However, the decrease in MRI activity appeared unrelated with the immunophenotypic features described above, evaluated 2 years after initiating IFNβ therapy (data not shown).

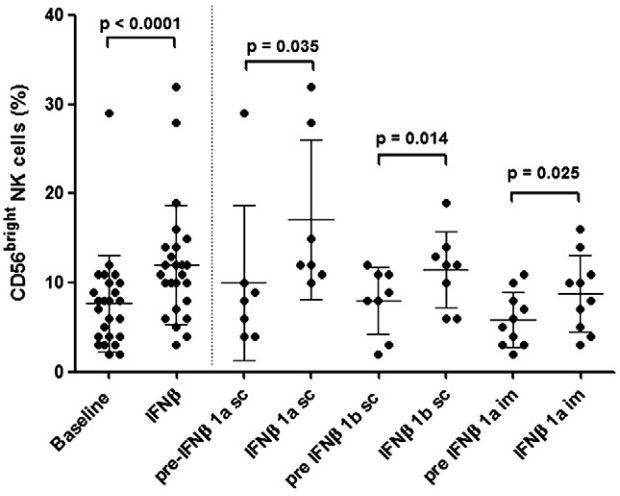

Figure 2 CD56^{bright} NK cells and IFNβ type analyzed at baseline and after 2 years of treatment. Analysis of the whole group of MS patients (n=25) is depicted on the left side of the figure, whereas the percentage of CD56^{bright} NK cells according to the IFNβ type (1a sc (n=7), 1b sc (n=8) and 1a im (n=10)) is shown on the right side. p Value: Wilcoxon signed rank test.

3.4. Expansion of CD56^{bright} NK Cells and NKR Expression in Responders and Non-responders after IFNβ Therapy

IFNβ therapy in clinical responders was associated with a statistically significant expansion of CD56^{bright} NK cells (magnitude of mean increase (Δ)=1.75 (1.04); $p=0.0007$). In contrast, the magnitude of change in the percentage of CD56^{bright} NK cells in non-responders was smaller (Δ=1.43 (0.59); $p=0.058$) (Fig. 3).

An analysis of NKR expression by NK cells according to the clinical response to IFNβ, revealed that responders had higher proportions of NKG2A+NK cells ($p=0.0002$) as compared to non-responders ($p=0.108$); responders had also lower KIR expression after IFNβ ($p=0.048$) as compared to non-responders ($p=0.865$) (Table 2, Fig. 3). By contrast, LILRB1 expression decreased after IFNβ in both responders and non-responders ($p=0.006$ and $p=0.042$, respectively) (Table 2, Fig. 3). Moreover, the age-dependent increase of LILRB1 expression by CD8+ T lymphocytes noted at baseline vanished after IFNβ independently of the clinical response (data not shown). A more detailed analysis of NKR expression by CD56^{bright} NK cells did not reveal significant differences between both groups of patients after 2 years of IFNβ therapy (data not shown).

4. Discussion

In the present study, we found that the clinical response to IFNβ therapy in MS patients appears related to the degree of expansion of CD56^{bright} NK cells in peripheral blood. Moreover, NKR expression (i.e., LILRB1, NKG2A, KIRs) by NK cells was related to the clinical response to this therapy, suggesting that NK cell dynamics and function may be associated with the immunological processes underlying to the

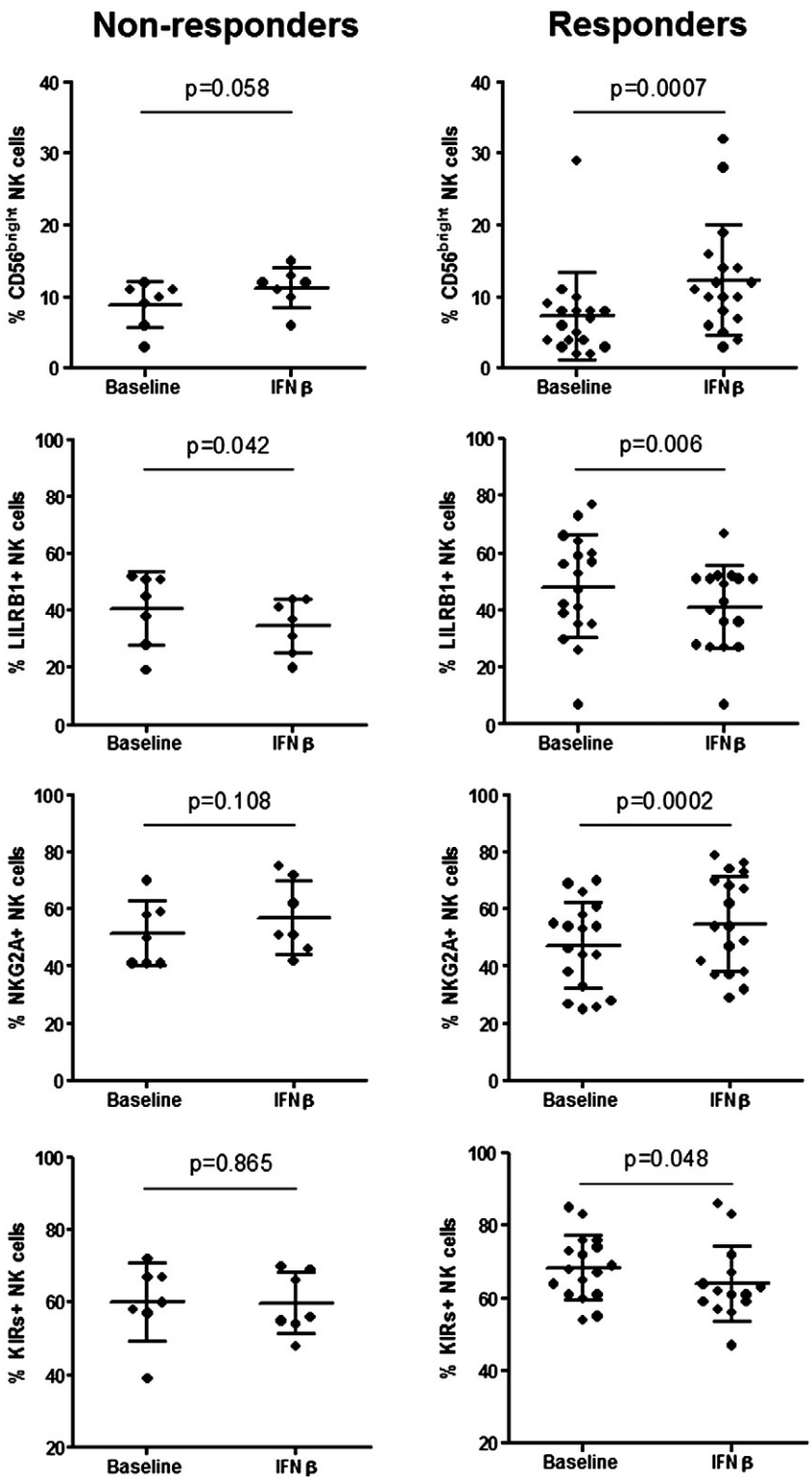

Figure 3 CD56^{bright} NK cells and NKR expression according to the response to IFNβ. Figures are depicted as box plot graphs of CD56^{bright} NK cells and NKR expression (LILRB1, NKG2A, KIRs) by NK cells before and after IFNβ treatment in clinical responders and non-responders. p Value: Wilcoxon signed rank test.

mechanism of action of IFNβ in MS. However, the baseline evaluation of NKR expression was not predictive of the clinical response to IFNβ, as both responders and non-responders displayed a similar immunophenotype at that stage.

CD56^{bright} NK cells are currently considered to give rise to CD56^{dim} NK cells, since the former have longer telomeres and appear first after hematopoietic stem cell transplantation [26]. In MS, previous studies have confirmed that the absolute numbers of CD56^{bright} NK cells increase in patients

treated with daclizumab and IFNβ, concomitantly with a decrease in the number of CD56^{dim} NK cells [9,12]. CD56^{bright} NK cells are the predominant NK cell subset in secondary lymphoid tissues, where they may exert important immunoregulatory functions and control of EBV infection [9,27], a virus related with MS pathophysiology. The mechanisms underlying the CD56^{bright} NK cell expansion, detected after 1–4 months of immunomodulatory therapies [12,28], are only partially understood [9,29]. The action of daclizumab has been related to a blockade of the human IL-2 receptor in T lymphocytes, leading to a higher bioavailability of IL-2 and expansion of NK cells [16,30]. By contrast, the mechanism of expansion of CD56^{bright} NK cells induced by IFNβ is unclear and might be related with the enhancement of NK cell functions and induction of an antiviral state [8].

In MS, the expansion of CD56^{bright} NK cells has been related with a positive response to daclizumab [9,28]; yet, whether this effect actively contributes to the response or is just an epiphenomenon is uncertain. We found an increase of CD56^{bright} NK cells in the majority of IFNβ-treated MS patients, though to a lower extent than that reported in daclizumab-treated patients, where an expansion of CD56^{bright} NK cells higher than 300% has been described in full responders [28]. In our IFNβ-treated MS patients, we observed a mean increase of CD56^{bright} NK cell expansion of 77% (91% in responders and 43% in non-responders). Altogether, these findings suggest that the NK cell expansion may be related to the therapeutic benefit of IFNβ.

In the present study, we also found modifications of the expression of some NKR in MS patients associated to IFNβ therapy. LILRB1 is an inhibitory receptor specific for HLA class I molecules that regulates leukocyte responses to different triggering stimuli, presumably contributing to control inflammatory responses [22]. LILRB1 has been reported to be expressed by CD8+ T lymphocytes at late differentiation stages and tends to increase in relation with age, reflecting an accumulation of effector/memory T cells [31]. We previously described that MS patients have higher proportions of LILRB1+ CD8+ T lymphocytes in relation to age and MS evolution as compared to controls [11]. In the present study, MS patients who did not respond to IFNβ therapy had at baseline a higher age-dependent LILRB1 expression as compared to responders, a finding that may be related to an accumulation of terminally differentiated cells in non-responders. Nevertheless, after IFNβ therapy, the age-related LILRB1 expression faded in non-responders as well as in responders, probably due to a decrease of peripheral effector/memory lymphocytes independently of the response to the treatment.

In a case–control analysis, IFNβ therapy was associated with higher NKG2A and lower LILRB1 and KIR expression by NK cells; no other differences were found comparing controls and untreated MS patients, thus suggesting a direct effect of IFNβ on NKR expression by NK cells. A further prospective analysis of patients followed-up after 2 years of IFNβ therapy showed that this pattern of NKR expression was related to a positive response to IFNβ. In a recent study, CD57 expression, a marker related with cellular senescence, was associated to a lower expression of NKG2A and progressive acquisition of KIRs by CD56^{dim} NK cells, contributing to define different stages of differentiation of the CD56^{dim} NK cell subset [14]. In this context, our results suggest that IFNβ therapy may promote a shift in the immunophenotypic profile of the NK cell compartment that is mainly found in responders, leading to an increase of cells at early differentiation stages. Whether daclizumab-treated patients may present similar changes of the NK cell immunophenotypic profile deserve further studies.

In conclusion, our results suggest that IFNβ may expand NK cell subsets with immunoregulatory functions and promote early maturation stages of this lymphocytic lineage mainly in those patients with a positive response to this therapy. Further studies focused on the mechanisms underlying the putative role of NK cells in MS may reveal novel biological pathways useful for diagnosis and therapy.

Supplementary materials related to this article can be found online at: doi:10.1016/j.clim.2011.09.006.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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