Seasonal variation in immune measurements and MRI markers of disease activity in MS

J. Killestein, MD; M.H.G. Rep, PhD; J.F. Meilof, MD, PhD; H.J. Adèr, PhD; B.M.J. Uitdehaag, MD, PhD; F. Barkhof, MD, PhD; R.A.W. van Lier, MD, PhD; and C.H. Polman, MD, PhD

Abstract—*Background:* The exact mechanisms by which T cells contribute to MS progression are not known. Recently, the results of cross-sectional studies suggested seasonal variation of both interferon (IFN)-γ production and the number of active MRI lesions in MS. *Objective:* To investigate whether seasonal fluctuations of IFN-γ and active MRI lesions could be confirmed and whether any correlations could be detected. *Methods:* Data were analyzed from a group of 28 MS patients in whom detailed longitudinal monitoring of both immune function and MRI measurements had taken place. *Results:* Significant seasonal variation was observed in T-cell activation as measured by the ability of T cells to secrete the pro-inflammatory cytokines tumor necrosis factor-α and IFN-γ. Maximum values were found in samples obtained during autumn. Even though clear fluctuations were observed, no significant seasonal variation could be detected in the number of active MRI lesions. Fluctuations of in vitro IFN-γ secretion correlated weakly with changes in active MRI lesions. *Conclusion:* The finding of seasonal variation of immune function in serially MRI-monitored MS patients suggests an environmental role in T-cell activation.

NEUROLOGY 2002;58:1077–1080

Evidence suggests that MS is the result of a complex interplay among several genes and currently unknown environmental factors. It is clear, however, that T cell-mediated inflammatory events in the CNS of patients with MS play a role in the pathogenesis of this disease. After priming, $CD4^+$ T-helper (Th) cells can mature into functionally different subsets, which can be distinguished by their cytokine secretion profile.[1] A distinction can be made between cytokines with pro-inflammatory properties, such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, produced by Th1 cells, and cytokines with anti-inflammatory properties, like interleukin (IL)-4 and IL-10, produced by Th2 cells.[2] Disease activity in the initial stage of MS may be dependent on activation of Th1-type cells that is insufficiently counterbalanced by Th2-type cells.[3] There is some evidence that IFN-γ and TNF-α promote disease activity in MS[4-7] and that IL-4 and IL-10 attenuate the disease process.[7-10]

Seasonal variation in cytokines and cell-mediated immunity has been found in animal studies,[11] in healthy volunteers,[12] and in MS patients.[13] Increased IFN-γ production has been reported in a small group of patients with progressive MS sampled in autumn and winter compared with other patients sampled in spring and summer.[13]

Seasonal fluctuations have also been reported for the prevalence of clinical exacerbations in MS. However, the course of these changes in MS activity varied between different geographic regions. For example, the peak exacerbation rate in relapsing-remitting MS was observed in the July through October period in northeastern Ohio,[14] in the winter and spring months in Switzerland,[15] and in the warmer months in Arizona.[16] Recently, further support for the view that season may influence (subclinical) disease activity has been provided by a study that showed a striking annual variation in the number of active MRI lesions.[17]

We analyzed data from MS patients, in whom detailed longitudinal monitoring had taken place, for seasonal fluctuations of both active MRI lesions and cytokine profiles.

Patients and methods. *Patients.* Analyses were carried out on 28 patients (table 1), who were enrolled (between January and December 1993) at the Department of Neurology, VU Medical Center, Amsterdam, the Netherlands, in a multicenter, randomized, double-blind, placebo-controlled, exploratory, phase II trial of the CD4 monoclonal antibody (mAb) cM-T412.[18] Because neither T-cell proliferation and cytokine production nor the number of active MR lesions was significantly affected by treatment with anti-CD4,[19] patients from both treatment arms were included in this study.

From the Department of Neurology (Drs. Killestein, Meilof, Uitdehaag, and Polman), MS-MRI Center (Dr. Barkhof), and Department of Clinical Epidemiology and Biostatistics (Drs. Adèr and Uitdehaag), VU Medical Center, and Department of Immunobiology (Drs. Killestein, Rep, and van Lier), CLB, Amsterdam, the Netherlands.

Received February 2, 2001. Accepted in final form December 18, 2001.

Address correspondence and reprint requests to Dr. J. Killestein, Department of Neurology, VU Medical Center, P.O. Box 7057, 1007 MB, Amsterdam, the Netherlands; e-mail: J.Killestein@vumc.nl

Table 1 *Baseline characteristics*

Variable	Value
Mean ± SD age, y	35.2 ± 7.5
Sex, n	
Male	13
Female	15
Disease type, n	
Relapsing-remitting	15
Secondary progressive	13
Disease duration, y, mean ± SD	4.2 ± 5.2
EDSS at inclusion, mean ± SD	5.0 ± 1.2

EDSS = Expanded Disability Status Scale.

Clinical and MRI examinations. As reported,[18] clinical examination including Expanded Disability Status Scale and MRI were performed at monthly intervals for 9 months and thereafter at months 12 and 18 (12 scans/patient). Active lesions were defined as lesions that showed new gadopentetate dimeglumine (Gd-DTPA) enhancement on T1-weighted images and as enlarging or new lesions on T2-weighted images that were not seen on Gd-DTPA-enhanced T1-weighted images. At each visit, blood was collected (12 samples/patient during 18-month follow-up). Peripheral blood mononuclear cells (PBMC) were isolated and cryopreserved immediately. To minimize interassay variability, samples from all time points from individual patients were analyzed in one run. PBMC (25×10^5 cells/mL) were cultured and stimulated in triplicate cultures with CD2 mAb in the presence of CD28 mAb, as previously reported.[19] Cells cultured without stimuli served as negative controls. The proliferative response (cpm) was measured after 4 days of culture by means of incorporation of [^{3}H]thymidine; 0.4 mCi/well of [^{3}H]thymidine was added 24 hours before harvesting, as previously described.[20] Cytokine production was measured using standard reagents.[19-22] The in vitro cytokine secretion of PBMC (IFN-γ, TNF-α, IL-4, and IL-10) was measured after 72 hours of culture by specific ELISA (CLB, Amsterdam, the Netherlands). Intracellular cytokine production was measured using 0.5×10^6 cells/mL, stimulated for 4 hours with phorbol myristate acetate and ionomycin in the presence of monensin.[19] After cell surface staining with CD3-fluorescein isothiocyanate, cytoplasm was stained with biotinylated cytokine mAb (IL-4, IFN-γ) followed by streptavidin–phycoerythrin. Cells were analyzed using a fluorescence-activated cell sorter (FACS; Becton Dickinson, Sunnyvale, CA).

Statistical analysis. Immunologic, clinical, and MRI data were pooled per season according to Balashov et al.[13] (March, April, May = spring; June, July, August = summer; September, October, November = autumn; December, January, February = winter). Repeated-measurement multivariate analysis of variance was used to evaluate within-subject changes over the four seasons. Anti-CD4 treatment, IV methylprednisolone (IVMP), and season of enrollment were entered as covariates. Correlations were generated using repeated-measurement regression analysis. To establish a normal distribution of the data, a log transformation was performed when data were nonnormally distributed (in vitro IL-4 and IL-10 secretion). To account for the multiple comparisons performed, a probability value of <0.01 was considered to be significant and <0.10 to indicate a trend.

Results. *Immune measurements.* Both in vitro TNF-α and IFN-γ secretion showed significant seasonal variation. Maximum values were found in samples obtained during autumn (TNF-α: $F_{1,27\ (quadratic)} = 9.0$, $p = 0.005$; and IFN-γ: $F_{1,27\ (quadratic)} = 9.4$, $p = 0.006$; tables 2 and 3). No significant seasonal variation was found in IL-4 or IL-10 secretion. A trend was observed for the percentage of T cells producing IFN-γ, as measured by flow cytometry. Again, maximum values were found in samples obtained during autumn ($F_{1,27\ (quadratic)} = 4.3$, $p = 0.047$; see tables 2 and 3). No significant seasonal variation was found in the percentage of T cells producing IL-4. No significant seasonal variation was found in T-cell proliferation. Neither active treatment nor season of enrollment was shown to have a significant impact on changes in immune measurements.

Clinical and MRI-documented disease activity. Even though clear fluctuations were observed, no significant seasonal variation could be detected in active MRI lesions (see tables 2 and 3) or in the number of patients experiencing clinical relapses (spring, six patients; summer, eight patients; autumn, nine patients; and winter, eight patients).

Table 2 *Mean (SD) values per season*

Measure	Spring	Summer	Autumn	Winter
T-Cell proliferation, cpm	23,566 (13,589)	26,192 (11,173)	30,249 (11,611)	28,951 (13,850)
In vitro TNF-α secretion, ng/mL	4.18 (3.68)	6.08 (5.66)	8.13 (8.4)	6.76 (7.30)
In vitro IFN-γ secretion, pg/mL	120 (117)	182 (131)	227 (206)	161 (104)
In vitro IL-4 secretion, pg/mL	255 (326)	292 (471)	275 (406)	294 (419)
In vitro IL-10 secretion, pg/mL	2,493 (3,875)	2,984 (4,176)	3,388 (3,913)	2,575 (2,697)
IFN-γ-producing T cells, %	21.4 (14.0)	23.7 (16.7)	27.8 (16.9)	24.6 (16.0)
IL-4-producing T cells, %	2.78 (1.70)	2.77 (1.88)	3.48 (2.8)	3.01 (1.95)
Mean no. of active MRI lesions	1.03 (1.81)	1.79 (3.60)	1.46 (2.32)	1.18 (2.23)
Patients with active MRI scan, %	44 (50)	53 (50)	52 (50)	49 (50)

TNF = tumor necrosis factor; IFN = interferon; IL = interleukin.

Table 3 *Mean changes (95% CI) per season*

Measure	Delta spring vs summer	Delta summer vs autumn	Delta autumn vs winter	Delta winter vs spring	$F_{quadratic}$	*p* Value
T-Cell proliferation, cpm	2,626 (−2,545/7,797)	4,057 (−1,882/9,996)	−1,298 (−6,914/4,318)	−5,385 (−10,937/167)	2.9	0.101
In vitro TNF-α secretion, ng/mL	1.90 (0.02/3.78)	2.05 (−0.98/5.08)	−1.37 (−3.14/0.40)	−2.58 (−4.01/−1.15)	9.9	0.005
In vitro IFN-γ secretion, pg/mL	62 (1/123)	45 (−9/99)	−66 (−133/1)	−41 (−92/10)	9.4	0.006
In vitro IL-4 secretion, pg/mL	37 (−98/172)	−17 (−111/77)	19 (−39/77)	−39 (−145/67)	0.1	0.762
In vitro IL-10 secretion, pg/mL	491 (−489/1,471)	404 (−880/1,688)	−813 (−1,639/−13)	−82 (−1,251/1,087)	1.5	0.230
IFN-γ-producing T cells, %	2.3 (−0.2/4.8)	4.1 (−1.3/9.5)	−3.2 (−7.2/0.8)	−3.2 (−7.0/0.6)	4.3	0.047
IL-4-producing T cells, %	−0.01 (−0.70/0.68)	0.71 (−0.44/1.86)	−0.47 (−1.63/0.69)	−0.23 (−1.45/0.99)	1.5	0.230
Mean no. active MRI lesions	0.76 (−0.45/1.97)	−0.33 (−1.54/0.88)	−0.28 (−1.30/0.74)	−0.15 (−1.03/0.73)	1.8	0.180
Patients with active MRI scan, %	9 (−3/21)	−1 (−19/17)	−3 (−26/20)	−5 (−29/19)	1.2	0.283

Significant quadratic trends over four seasons of in vitro tumor necrosis factor (TNF)-α and interferon (IFN)-γ secretion and percentage of IFN-γ-producing T cells in 28 longitudinally studied MS patients (repeated-measurement multivariate analysis of variance).

IL = interleukin.

Correlations between immune measurements and active MRI lesions. No significant correlations could be found between fluctuations in immune measurements and active MRI lesions. However, a trend was observed for the correlation between changes of in vitro IFN-γ secretion and active MRI lesions ($r = -0.354$, $p = 0.082$; table 4).

Discussion. We present the first study in which a group of patients with relapsing-remitting and secondary progressive MS was analyzed longitudinally for seasonal variations in both immunologic and MRI markers of disease activity.

In agreement with other studies, an annual rhythm of pro-inflammatory cytokine secretion patterns was observed.[13] In line with our findings, seasonal variation of the IFN-γ-producing capacity, with a decrease in summertime, has been reported in healthy Finnish subjects.[12] Furthermore, Balashov et al.[13] recently reported, in a cross-sectional study, maximum IFN-γ production in samples obtained during autumn, which is also in agreement with our findings. IFN-γ has been directly implicated as participating in the disease process based on the observation that when given to MS patients, systemic IFN-γ increases the frequency of clinical relapses.[4] Increased production of IFN-γ before a clinical relapse has been demonstrated by some,[23] whereas others found no clear relation between disease activity and IFN-γ secretion.[5-7]

Table 4 *Correlations between immune measurements and active MRI lesions over four seasons*

Immune measurement	*r*	*p* Value
T-Cell proliferation, cpm	−0.212	0.289
In vitro TNF-α secretion, ng/mL	−0.253	0.203
In vitro IFN-γ secretion, pg/mL	−0.354	0.082
In vitro IL-4 secretion, pg/mL	0.053	0.799
In vitro IL-10 secretion, pg/mL	0.013	0.948
IFN-γ-producing T cells, %	−0.296	0.150
IL-4-producing T cells, %	−0.133	0.526

No significant correlations between immune measurements and active MRI lesions over seasons were observed. However, a trend was observed for the correlation between changes of in vitro IFN-γ secretion and active MRI lesions ($r = -0.354$, $p = 0.082$). The correlations were generated using repeated-measure regression analysis (quadratic trends) in 28 longitudinally studied MS patients.

TNF = tumor necrosis factor; IFN = interferon; IL = interleukin.

Although the mean number of enhancing MRI lesions clearly fluctuated, we could not confirm the existence of significant seasonal variation in active MRI lesions. Two other studies have recently addressed the issue of seasonal effects of MRI disease activity in MS.[17,24] The first study reported that the frequency and extent of active lesions on MRI are influenced by seasonal fluctuations and are significantly higher in the first than in the second half of the year.[17] This study, however, can be criticized because longitudinal follow-up within patients was limited and variable. The second study also reported that the number of active lesions varied in the different seasons but that these variations were not statistically significant.[24] It has been argued that this absence of a statistically significant variation could be attributed to selection of patients in a very active phase of the disease (with subsequent "regression to mean") or the inclusion of patients from different geographic locations.[25] Our findings, the magnitude of fluctuations not being statistically significant, are in line with the second study,[24] even though our patient sample originates from one well-defined geographic area and covariate analysis showed that regression to the mean was not observed.

With use of repeated-measurement regression analysis, a trend for a weak negative correlation was found between changes of in vitro IFN-γ-secreting ability by PBMC and fluctuations in MRI-documented disease activity. The fact that most regression coefficients are negative (see table 4) is

perfectly in line with a previously published study in which active MRI lesion appearance seemed to be preceded by a decrease in circulating cytokine-producing T cells.[26]

It should be noted here, however, that in this exploratory study, the observed alterations in immune measurements are relatively modest and that the *p* values indicate the significance of quadratic trends in the mean values over time, not necessarily that the mean values are significantly higher during autumn than during other seasons. Furthermore, it should be noted that the quadratic shape was not prespecified. Therefore, our *p* values need to be interpreted with caution.

Seasonal cycles of disease prevalence and immune function exist in avian and mammalian populations.[11] In wild populations of rodents, lymphatic organ size and immune function increase in the fall and winter and then decline in the spring and summer.[11] The effects of season on the immune system are considered to be an adaptive or survival mechanism to help animals cope with the more physiologically demanding winter environment (e.g., decreased temperature, increased energy needs, decreased food availability, and increased stress).[11]

Seasonal rhythms can result both from cycles in immune competence and therefore susceptibility to disease and from differences in exposure to pathogenic organisms.[11] Viral infections are more common in particular seasons and have been shown to increase the relative risk for relapse.[14] Therefore, viral infection rates have been suggested as a possible explanation of the observed fluctuations of immunologic and radiologic effects in MS.[13,17,27] However, our patient group was not systematically studied for viral and other infections during the trial.

In our study, 30 of the 41 relapses were treated with IVMP. No significant differences between IVMP-treated and nontreated patients could be demonstrated, thus indicating that IVMP administration does not account for the seasonal variation in immune measurement changes. Furthermore, no significant differences could be demonstrated between patients with relapsing–remitting and secondary progressive MS. In addition, no differences were found between anti-CD4- and placebo-treated patients, as could be anticipated based on the results of the previously published analysis of this trial.[19]

References

1. Bottomly K. A functional dichotomy in $CD4^+$ T lymphocytes. Immunol Today 1988;9:268–274.
2. Romagnani S. Human Th1 and Th2 subsets: doubt no more. Immunol Today 1991;12:256–257.
3. Charlton B, Lafferty KJ. The Th1/Th2 balance in autoimmunity. Curr Opin Immunol 1995;7:793–798.
4. Panitch HS, Hirsch RL, Haley AS, Johnson KP. Exacerbations of multiple sclerosis in patients treated with gamma interferon. Lancet 1987;1:893–895.
5. Van Oosten BW, Barkhof F, Scholten PE, et al. Increased production of tumor necrosis factor alpha, and not of interferon gamma, preceding disease activity in patients with multiple sclerosis. Arch Neurol 1998;55:793–798.
6. Rieckmann P, Albrecht M, Kitze B, et al. Tumor necrosis factor-alpha messenger RNA expression in patients with relapsing–remitting multiple sclerosis is associated with disease activity. Ann Neurol 1995;37:82–88.
7. Van Boxel-Dezaire AHH, Hoff SCJ, Van Oosten BW, et al. Decreased interleukin-10 and increased interleukin-12p40 mRNA are associated with disease activity and characterize different disease stages in multiple sclerosis. Ann Neurol 1999;45:695–703.
8. Salmaggi A, Dufour A, Eoli M, et al. Low serum interleukin-10 levels in multiple sclerosis: further evidence for decreased systemic immunosuppression? J Neurol 1996;243:13–17.
9. Rieckmann P, Albrecht M, Kitze B, et al. Cytokine mRNA levels in mononuclear blood cells from patients with multiple sclerosis. Neurology 1994;44:1523–1526.
10. Link J, Soderstrom M, Olsson T, et al. Increased transforming growth factor-β, interleukin-4, and interferon-γ in multiple sclerosis. Ann Neurol 1994;36:379–386.
11. Mann DR, Akinbami MA, Gould KG, Ansari AA. Seasonal variations in cytokine expression and cell-mediated immunity in male rhesus monkeys. Cell Immunol 2000;200:105–115.
12. Katila H, Cantell K, Appelberg B, Rimon R. Is there a seasonal variation in the interferon-producing capacity of healthy subjects? J Interferon Res 1993;13:233–234.
13. Balashov KE, Olek MJ, Smith DR, et al. Seasonal variation of interferon-α production in progressive multiple sclerosis. Ann Neurol 1998;44:824–828.
14. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. Lancet 1985;1:1313–1315.
15. Wutrich R, Rieder HP. The seasonal incidence of multiple sclerosis in Switzerland. Eur Neurol 1970;3:157–164.
16. Bamford CR, Sibley WA, Thies C. Seasonal variation of multiple sclerosis exacerbations in Arizona. Neurology 1983;33:897–907.
17. Auer DP, Schumann EM, Kumpfel T, et al. Seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. Ann Neurol 2000;47:276–277.
18. Van Oosten BW, Lai M, Hodgkinson S, et al. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. Neurology 1997;49:351–357.
19. Rep MH, van Oosten BW, Roos MT, et al. Treatment with depleting CD4 monoclonal antibody results in a preferential loss of circulating naive T cells but does not affect IFN-gamma secreting TH1 cells in humans. J Clin Invest 1997;99:2225–2231.
20. Rep MH, Hintzen RQ, Polman CH, van Lier RA. Recombinant interferon-beta blocks proliferation but enhances interleukin-10 secretion by activated human T-cells. J Neuroimmunol 1996;67:111–118.
21. Rep MH, Schrijver HM, van Lopik T, et al. Interferon (IFN)-beta treatment enhances CD95 and interleukin 10 expression but reduces interferon-gamma producing T cells in MS patients. J Neuroimmunol 1999;96:92–100.
22. Van der Meide PH, Dubbeld M, Schellekens H. Monoclonal antibodies to human immune interferon and their use in a sensitive solid-phase ELISA. J Immunol Methods 1985;79:293–305.
23. Beck J, Rondot P, Catinot L, et al. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? Acta Neurol Scand 1988;78:318–323.
24. Rovaris M, Comi G, Sormani MP, et al. Effects of seasons on magnetic resonance imaging–measured disease activity in patients with multiple sclerosis. Ann Neurol 2001;49:415–416.
25. Tremlett H. Effects of seasons on magnetic resonance imaging–measured disease activity in patients with multiple sclerosis. Ann Neurol 2001;50:422–423.
26. Killestein J, Rep MH, Barkhof F, et al. Active MRI lesion appearance in MS patients is preceded by fluctuations in circulating T-helper 1 and 2 cells. J Neuroimmunol 2001;118:286–294.
27. Edwards S, Zvartau M, Clarke H, Irving W, Blumhardt LD. Clinical relapses and disease activity on magnetic resonance imaging associated with viral upper respiratory tract infections in multiple sclerosis. J Neurol Neurosurg Psychiatry 1998;64:736–741.