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Role of peripheral CD8 lymphocytes and soluble IL-2 receptor in predicting the duration of corticosteroid treatment in polymyalgia rheumatica and giant cell arteritis

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Abstract

***Objectives*—To determine if the presence of low percentages of CD8 positive cells or high levels of soluble interleukin-2 receptors (sIL-2R) define a subgroup of patients with more severe polymyalgia rheumatica and giant cell arteritis (PMR/GCA).**

***Methods*—38 PMR/GCA patients were followed up prospectively. Serum levels of sIL-2R and peripheral blood CD8 lymphocytes were measured before the start of corticosteroid treatment, after six months of treatment and at the last visit. Phenotypical analysis of lymphocyte subpopulations was performed with a two colour technique, and assay of sIL-2R was performed using an enzyme-linked immunosorbent kit. Forty four healthy people matched for age and gender comprised a healthy control group.**

***Results*—The median duration of follow up was 28 months (range 7–65). Corticosteroid treatment lasted a median of 23·5 months (7–65). Eleven patients (29%) were in remission at the end of follow up; 45% of the patients had at least one relapse or recurrence. Compared with controls, patients with active disease had a significantly lower percentage of CD8 cells and significantly increased sIL-2R levels. Erythrocyte sedimentation rate, C reactive protein, and sIL-2R values were significantly less after six months of steroid treatment compared with before treatment. The percentage of CD8 cells remained significantly lower at six months and the end of follow up compared with controls, while sIL-2R levels remained significantly greater. Patients in whom the percentage of CD8 cells at six months was lower than one SD of the mean of normal controls (26%) had a significantly longer duration of corticosteroid treatment, a greater cumulative dose of prednisone and more relapses or recurrences compared with patients in whom the percentage was in the normal range. The duration of treatment and the cumulative dose of prednisone were not influenced by the percentage of CD8 cells before treatment therapy or by the levels of sIL-2R after six months of treatment.**

***Conclusions*—A reduced percentage of CD8 cells after six months of treatment may be a useful outcome parameter which would identify a group of PMR/GCA patients likely to experience more severe disease, defined as longer duration of corticosteroid treatment, higher cumulative dose of prednisone, and relapse or recurrence of disease.**

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There are conflicting data on the duration of corticosteroid treatment in polymyalgia rheumatica with or without temporal arteritis (PMR/GCA), but some studies have identified a population of PMR/GCA patients with persistent disease which requires long term steroid treatment (4–5 years);[1–5] some of these patients may need low doses of corticosteroid indefinitely. The persistence of active disease in PMR/GCA is associated with the presence of relapse or recurrence of disease when corticosteroid treatment is reduced or stopped. The duration of corticosteroid treatment is related to the number of relapses or recurrences, and it is a good indicator of disease severity, but there are no reliable predictors of disease severity and duration of corticosteroid treatment in PMR/GCA patients.

It has been shown that some immunological factors, such as CD8 positive cells,[6–8] soluble interleukin-2 receptors (sIL-2R),[9] interleukin-6,[10 11] serum soluble CD8,[12] and serum soluble intercellular adhesion molecule-1,[13] are associated with disease activity. Levels of these factors are altered in active disease and return to normal after corticosteroid treatment, along with clinical remission of the disease. However, some longitudinal studies demonstrated the persistence of increased sIL-2R[9] and of CD8 cell depletion[6 8] in some patients after months of corticosteroid treatment and clinically inactive disease.

We studied a series of patients prospectively to evaluate whether the presence of low percentages of CD8 cells, high levels of sIL-2R, or both, defined a subgroup of patients with more severe disease. PMR/GCA severity was evaluated on the basis of duration of corticosteroid treatment, cumulative prednisone dose, and relapse or recurrence of disease.

Patients and methods

Thirty eight patients with PMR (according to the criteria suggested by Healey[14]) were investigated. Table 1 shows their clinical and demographic data. A temporal artery biopsy specimen was obtained only in patients with cranial signs or symptoms and revealed GCA in three patients. All patients were assessed clinically at presentation, monthly for the first six months, then every three months, by the same physician (CS, FR, or PM) during the follow up period. Rheumatological assessment included evaluation of systemic symptoms (fever, anorexia and weight loss), cranial and ocular signs and symptoms, and duration of morning stiffness. Blood samples were obtained at the time of disease diagnosis (before steroid treatment), after six months of corticosteroid treatment, and at the last visit.

Erythrocyte sedimentation rate (ESR) and a complete routine laboratory assessment were performed using standard laboratory methods. C reactive protein (CRP) was measured by nephelometry (NA latex CRP kit, Behringwerke, Marburg, Germany) (upper limit of the normal reference range 5 mg/l). Serum sIL-2R levels were measured using a sandwich enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (T Cell Science, Cambridge, MA, USA). Phenotypic analysis of lymphocyte subpopulations was performed with a two colour technique using association of specific monoclonal antibodies (Becton-Dickinson, Sunnyvale, CA, USA). Flow cytometry was performed with a FACSCAN 440 machine (Becton Dickinson) with a single argon laser (wave length 488 nm).

Forty four healthy subjects matched for age and gender were used as the control group; 41 of them were used as control group for sIL-2R measurements. Blood of the patients and controls was taken at the same time and phenotypic analyses of lymphocytes performed within two hours.

Relapse and recurrence were considered present if signs or symptoms occurred (usually with an ESR greater than 30 mm/1st h) in a patient receiving corticosteroids or after discontinuation of treatment.

Patients with PMR received prednisone at an initial daily dosage of 12·5 mg/day. The three patients with associated GCA received a starting dosage of prednisone 50 mg/day, modified subsequently to achieve the smallest dose capable of controlling the disease.

The medical records of all patients were reviewed and the cumulative prednisone dose computed; in addition, the weighted average dose during the first six months of treatment was estimated according to the following formula:

$$\text{weighted average dose during an interval} = \text{dose 1} \times \frac{(\text{estimated number of days})}{(\text{total number of days in interval})} + \text{dose 2} \times \frac{(\text{estimated number of days})}{(\text{total number of days in interval})} + \text{dose 3 ... etc.}$$

Friedman and Mann-Whitney tests were used for analysing data. Chi-square test with Fisher's correction was used when necessary. Correlations were assessed with Pearson's correlation coefficient.

Table 1 Characteristics of 38 patients studied

Female/male (%)	76/24
Age at onset of disease (years)	72·5 (54–84)
Duration of disease before diagnosis (months)	3·0 (1–6)
Duration of treatment (months)	23·5 (7–65)
Duration of follow up (months)	28·0 (7–65)
Systemic symptoms and signs (fever, anorexia, weight loss) (%)	17 (45%)
Morning stiffness (min)	150 (60–240)
Initial prednisone dose (mg/day)	20·8 (12·5–50)
Weighted average prednisone dose at six months (mg/day)	13·0 (7·4–41·9)
Cumulative prednisone dose (g)	5·6 (1·9–11·8)

Data expressed as median and range or percentage.

Table 2 Duration of treatment and follow up, and cumulative dose of prednisone in the patients with and without relapse or recurrence of disease

	At least one relapse/recurrence (n = 17)	*No relapse/recurrence (n = 21)*	*p*
Duration of treatment (months)	33 (15) 34 [7–65]	21 (9) 18 [8–42]	0·004
Duration of follow up (months)	33 (15) 34 [7–65]	27 (12) 24 [8–54]	NS
Cumulative prednisone dose (g)	6·8 (2·3) 7·0 [1·9–11·8]	4·7 (1·8) 4·4 [1·9–9·9]	0·003

Values are mean (SD) and median [range].

Table 3 Outcome measures in 38 patients with PMR and in 44 control patients

Outcome measure	*Baseline*	*Six months*	*Last visit*	*Control group*
ESR (mm/1st h)	79 (29) 74 [31–140]	20 (14)†††† 14 [5–53]	23 (20)†††† 19 [2–101]	—
CRP (mg/l)	61 (40) 54 [3–157]	8 (7)†††† 4 [2–34]	10 (13)†††† 5 [2–64]	—
sIL-2R (U/ml)§	859 (530)**** 684 [344–3200]	425 (196)††††*** 397 [127–898]	347 (184)††††* 313 [102–1023]	260 (49) 260 [170–510]
CD8+, CD4– (%)	28 (8)*** 29 [14–43]	29 (9)*** 27 [14–52]	30 (9)* 30 [10–47]	35 (9) 33 [21–53]

Values are mean (SD) and median [range].
ESR = Erythrocyte sedimentation rate; CRP = C reactive protein; sIL-2R = soluble interleukin-2 receptor.
§n = 41 for control group.
Significant differences: ††††p = 0·0001 compared with baseline; *p = 0·02, ***p = 0·001, ****p = 0·0001 compared with healthy controls.

Results

Eleven patients (29%) were in remission at the end of the follow up period. In these patients, the median follow up after prednisone discontinuation was 12 months (range 5–34). At the last visit, 27 patients (71%) remained on prednisone treatment and 15 were taking a prednisone dose lower than 5 mg/daily; the other 12 patients were taking prednisone ≥5 mg/daily.

Twenty one patients (55%) did not have a relapse or recurrence of disease, 11 (29%) had one relapse or recurrence and six (16%) had at least two (table 2).

Table 3 shows that sIL-2R levels were significantly increased in patients with active PMR before treatment compared with healthy controls (p = 0·0001), while the percentage of CD8 cells was significantly smaller (p = 0·001). Pretreatment values of ESR, CRP

and sIL-2R decreased significantly after six months of steroid treatment. At the end of follow up, sIL-2R levels were significantly reduced compared with those at six months ($p = 0.03$), but values at both these measurements remained significantly greater than those in controls ($p = 0.001$ and 0·01, respectively). The percentage of CD8 lymphocytes after six months of treatment remained significantly less in patients than in controls ($p = 0.001$). At the end of follow up, it had increased (but not significantly) compared with the pretreatment and six month values, but remained significantly less than in controls ($p = 0.02$).

The pretreatment percentage of CD8 cells was less than one standard deviation (SD) of the mean of normal controls (cut off point <26%) in 15 of 38 patients (39%). The duration of treatment and the cumulative dose of prednisone were not influenced by the baseline values of CD8 cells (26 (SD 14) months for low levels compared with 26 (13) months for normal levels (NS); 5·4 (2·0) g compared with 5·7 (2·5) g (NS), respectively). Sixteen of 38 patients (42%) had a percentage of CD8 cells at six months which was less than 26% (table 4). Ten of these 16 patients also had low pretreatment percentages of CD8 cells; the remaining six had normal pretreatment percentages. Low levels of CD8 cells persisted at the end of follow up (last visit) in 10 of the 16 patients; seven of the 10 were receiving corticosteroid treatment at the last visit.

The 16 patients with low percentages of CD8 cells at six months had a longer duration of treatment and a higher cumulative dose of prednisone compared with patients with a percentage of CD8 cells in the normal range (32 (14) months compared with 22 (12) months ($p = 0.03$); 6·9 (2·4) g compared with 4·7 (1·8) g ($p = 0.005$), respectively). There were no statistically significant differences in duration of follow up in the two groups of patients (34 (12) months compared with 29 (14) months (NS)).

Excluding the 15 patients receiving a dose of prednisone less than 5 mg/day at the last visit, the duration of therapy was significantly longer in the nine patients with a low percentage of CD8 cells at six months compared with the 14 patients with normal values (35 (17) months compared with 19 (12) months ($p = 0.02$)). The cumulative dose of prednisone was also significantly greater in patients with low percentages of CD8 cells at six months (6·9 (3·0) g compared with 4·7 (2·0) g ($p = 0.05$)). There were no statistically significant differences in the duration of follow up between these two groups of patients (39 (12) months compared with 27 (15) months (NS)).

The percentage of patients with at least one relapse or recurrence of disease was significantly greater among the group with a low percentage of CD8 cells at six months compared with those with normal levels (65% *v* 24%; $p = 0.01$). No significant differences were found between the groups having low and normal percentages of CD8 cells before treatment (47% *v* 33%; NS).

sIL-2R values were found to be greater than two SD of the mean concentration of normal controls (cut off point >358 U/ml) at the pretreatment measurement in 36 of the 38 patients (95%) and at the six month follow up in 22 of the 38 (58%). High sIL-2R levels persisted at the end of follow up in 11 of the latter 22 patients; seven of these patients were receiving corticosteroid treatment at the last visit. There were no significant differences in duration of treatment or cumulative dose of prednisone between patients with sIL-2R levels higher, compared with lower, than two SD of the mean concentration of normal controls after six months of treatment (28 (15) months compared with 24 (11) months (NS); 6·0 (2·5) g compared with 5·2 (2·0) g (NS), respectively). These differences remained insignificant after the exclusion of 15 patients receiving less than 5 mg of prednisone daily at the end of follow up (data not shown).

Among the patients with high and normal sIL-2R levels after six months of treatment there were no significant differences in the percentage of patients with relapse or recurrence of their disease (47% *v* 67%; NS).

There were no correlations between the percentage of CD8 cells at six months and, respectively, the weighted average dose of prednisone during the first six months of treatment ($r = -0.09$; NS) and the actual dose of prednisone at six months ($r = -0.08$; NS).

Table 4 CD8 percentages and clinical data of 16 patients with six month low CD8 values

Patient	CD8 (%)			Duration (months)		Time after CS withdrawal (months)	Cumulative dose of prednisone (g)
	Baseline	Six months	Last visit	Treatment	Follow up		
1	32	25	30	54	54	0	11·9
2	28	21	24	39	39	0	9·9
3	30	21	33	24	40	16	4·6
4	25	19	23	36	36	0	6·6
5	22	24	24	22	34	12	3·0
6	14	16	10	38	38	0	5·7
7	29	25	30	42	42	0	7·0
8	30	25	31	34	34	0	7·9
9	18	24	35	29	29	0	7·3
10	17	16	18	14	20	6	4·0
11	17	19	24	22	22	0	4·7
12	15	14	16	18	24	6	4·4
13	17	17	18	27	27	0	8·3
14	15	16	18	20	20	0	7·1
15	35	22	28	23	23	0	5·4
16	24	16	16	65	65	0	9·2

CS = Corticosteroid.

Discussion

While we did not observe any differences in duration of corticosteroid treatment, cumulative dose of prednisone, and relapse or recurrence of disease in patients with low and normal percentages of CD8 cells before treatment, we did observe that a low percentage of CD8 cells at six months was related to a longer duration of corticosteroid treatment, a higher cumulative dose of prednisone, and relapse or recurrence of disease.

Other, longitudinal, studies have revealed the persistence of increased sIL-2R[9] and IL-6[10 11] values, and of CD8 cell depletion[6 8] in some patients after months of corticosteroid treatment. These studies indicate immunological activation in a subgroup of patients with PMR/GCA that persists for a much longer time, despite rapid control of disease manifestations by corticosteroids. The presence of two populations of PMR/GCA patients was suggested by some studies[3 15 16] which estimated the cumulative probability of requiring continued corticosteroid treatment by using life table methods. One population presented a mild, self limiting disease requiring short term treatment; the other had a persisting disease which required long term treatment. These studies[3 16] aimed to identify clinical features that may predict the different situations of treatment in PMR/GCA patients. However, analysis of various parameters failed to identify any outcome predictors. Our study is the only one which has aimed to determine if the presence of low percentages of CD8 cells or high sIL-2R levels define a subgroup of patients with more severe disease. The severity of PMR/GCA is related to the duration of corticosteroid treatment, which in turn is associated with the presence of relapse or recurrence of disease when corticosteroid treatment is reduced or stopped. Northern European studies[1 2 4] have suggested that corticosteroid treatment for PMR, GCA or both, may be necessary for at least two years. The cumulative dose of prednisone reflects the patient's exposure to this drug, and it may also be a good indicator of disease severity if a well defined procedure is used. Pountain *et al*[17] showed that prednisolone treatment causes a significant decrease in the percentage of CD8 cells, particularly in older healthy volunteers. These authors suggested that corticosteroids could be responsible for the T cell changes observed in PMR/GCA. The association which we have now observed between low percentages of CD8 cells and longer duration of prednisone treatment could, therefore, simply have reflected a greater prednisone dose requirement at six months; however, the absence of any correlation between the actual dose of prednisone at six months and the percentage of CD8 cells at the same time excludes this possibility. There was also no correlation between the six month weighted average dose of prednisone, which more closely reflects the patient's exposure to prednisone, and the percentage of CD8 cells at the same time.

A reduced percentage of CD8 cells after six months of treatment appears to define patients with more severe disease. However, the variability in the distribution of CD8 values and the small number of patients studied impose limitations on our results. Even if the majority of the patients with six month low CD8 values had received long term prednisone treatment, some patients with low levels did not appear to have more severe disease (table 4).

In common with the findings of previous studies,[6-8] we observed a decrease in the percentage of CD8 cells in active PMR/GCA compared with controls. While low CD8 values before treatment were observed in 39% of our patients, other studies[7 18] have shown a reduced percentage of CD8 cells in nearly 80% of patients with active untreated PMR/GCA, leading Arnold *et al*[18] to propose this parameter as a diagnostic criterion. However, Pountain *et al*[19] did not show reduced levels of CD8 in active PMR/GCA. Differences in patient selection and in techniques of evaluation of CD8 T cells may partially explain these different results.

High sIL-2R levels were not useful outcome parameters but, as in our previous study,[9] we confirmed the persistence of high sIL-2R levels despite apparent clinical remission of the disease.

In conclusion, these data suggest that a reduced percentage of CD8 cells after six months of treatment may be a useful outcome parameter to identify a group of PMR/GCA patients likely to experience a longer duration of corticosteroid treatment, higher cumulative dose of prednisone and relapse or recurrence of disease. Further multicentre prospective studies are required to confirm this observation and to evaluate the reasons for the differences in pretreatment CD8 levels observed in different studies.

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