

Circulating T cell subtypes in polymyalgia rheumatica and giant cell arteritis: variation in the percentage of CD8+ cells with prednisolone treatment

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Abstract

Objectives—Some reports have described a decreased percentage of circulating CD8+ cells in patients with polymyalgia rheumatica and giant cell arteritis (PMR/GCA) before treatment and persisting for some months during treatment with corticosteroids. Other studies have found no such changes. There are overt methodological variations between these studies and there may also be hidden differences, such as the timing of blood samples. The purpose of this study was to investigate T cell subtypes in patients with PMR/GCA while controlling for variables known to affect T cells.

Methods—Circulating T cell subsets were measured in 36 patients with PMR/GCA before and during treatment with prednisolone. Blood samples during treatment were taken before the daily dose of prednisolone. The whole blood lysis method was used followed by flow cytometry.

Results—Compared with controls, CD8+ cells were not reduced before treatment in patients with PMR/GCA ($0.44 \times 10^9/l$; 28% of lymphocytes). CD4+ cells were also normal ($0.78 \times 10^9/l$; 48% of lymphocytes). During treatment with prednisolone total T cells increased from 1.18 to $1.59 \times 10^9/l$ and CD4+ cells increased from 0.78 to $1.05 \times 10^9/l$. The percentage of CD8+ cells decreased on treatment from 28 to 25%.

Conclusions—This study does not confirm the finding of some groups that the percentage of circulating CD8+ cells is reduced in patients with PMR/GCA before treatment. It does show that the percentage of CD8+ cells decreases during treatment with corticosteroids. This needs to be considered when designing studies of lymphocyte subsets in diseases treated with corticosteroids.

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There have been several studies of circulating T cell subtypes in patients with polymyalgia rheumatica and giant cell arteritis (PMR/GCA) with differing results. In GCA before treatment, Andersson *et al*¹ found normal numbers of CD8+ and CD4+ cells, and Banks *et al*² documented normal ratios of helper to

suppressor cells. Other studies in patients with PMR and GCA have found a decreased percentage of CD8+ cells,³⁻⁷ although Dasgupta *et al*⁴ and Elling *et al*³ also reported reduced absolute numbers of CD8+ cells.

All of the studies apart from the negative study by Andersson *et al*¹ used mononuclear cells separated on a Ficoll-Hypaque density gradient. This method selectively decreases the CD8+ subset, leading to a significantly lower percentage of CD8+ cells than the whole blood lysis method.⁸ This artefact would not necessarily affect samples from controls and patients to the same extent, so could distort the results. Patient CD8+ cells might have intrinsic differences from control cells affecting their migration on a density gradient and might well also have differences due to a delay in processing compared with control cells. A marked decrease in the percentage of CD8+ cells and CD4+ cells has been shown in blood stored for 24 hours when the Ficoll-Hypaque method is used, but not with the whole blood lysis method.⁹ Furthermore, a delay of more than six hours before processing blood samples results in a considerable decrease in the absolute number of lymphocytes counted by automated haematology counters.¹⁰ Such a delay might occur more often with patient samples than control samples, particularly in multicentre studies where patient blood samples may be transported from other hospitals for analysis.

The diurnal variation in lymphocyte numbers produces a nadir in the number of lymphocytes, T cells, CD4+, and CD8+ cells around 1000¹¹ to 1230 hours.¹² Obviously, if patient and control samples are not taken at the same time of day, this would confound results.

There is evidence of a decrease in CD8+ cells with age, both in absolute number and as a percentage of lymphocytes,^{13 14} so controls should be matched for age, which is not addressed in some of the previous studies.^{1 2}

In some of the previous studies the patients with PMR/GCA were already receiving prednisolone at the time of T cell subset analysis.^{6 7} We have seen changes in T cell subtypes occurring with prednisolone in normal volunteers.^{14a} Blood samples taken seven hours after a single 20 mg dose of prednisolone have a lowered absolute number of CD8+ cells, whereas the effect of longer term treatment with prednisolone is to increase the absolute numbers of CD8+ cells. These effects parallel the changes in total lymphocyte numbers. CD8+ cells as a

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percentage of lymphocytes do not change significantly in the first few hours, but with longer term prednisolone treatment this percentage decreases significantly. Hence corticosteroid treatment alters the T cell subtypes depending on the timing of the sample.

To clarify the conflicting data from previous work, this study was designed to eliminate these variables.

Patients and methods

Thirty six patients with PMR/GCA were assessed before, during, and after treatment with prednisolone. Ages ranged from 51 to 87 years (median 70 years). Diagnosis was made according to the clinical criteria of Jones and Hazleman.¹⁵ Increased erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) were not used as inclusion criteria, as these and other laboratory variables were to be studied, but in fact 34 of 36 patients did have an ESR above 30 mm/h or a CRP above 6 mg/l, or both. Serum creatine kinase and protein electrophoresis were normal in all patients with PMR.

These patients had blood samples taken between 1000 and 1400 hours, before treatment, and subsequently after 10 days, three weeks, six weeks, and three months of treatment, and thereafter every three months until treatment could be discontinued. Venesection was always carried out at the same time of day and before the daily prednisolone dose. Treatment for PMR was begun with prednisolone 10–20 mg daily, or for GCA with prednisolone 40 mg daily. Prednisolone was prescribed as the enteric coated preparation and as a once daily dose in the morning. Subsequently the dose was reduced according to clinical disease activity.

Controls were matched for age and sex and consisted of healthy volunteers and patients with osteoarthritis. Ethical committee approval was obtained to approach the controls for blood tests. Control samples were collected between 1000 and 1400 hours.

All patients and controls were seen at Addenbrooke's Hospital and all blood samples were processed within six hours of venesection. The total white blood cell count and lymphocyte count were measured using routine techniques in the haematology department at Addenbrooke's Hospital. T cell subtypes were analysed by flow cytometry using a whole blood lysis technique. Total T cells were measured using antibodies to CD3 (Leu 4). Activated T cells were those CD3+ cells which coexpressed antibodies to HLA-DR. CD4+ cells were defined using Leu 3 and CD8+ cells using Leu 2. Natural killer cells were CD3- expressing CD 16/56 (Leu 11c+19). All monoclonal antibodies were purchased from Becton Dickinson (Oxford, United Kingdom) from the Simulset range. Aliquots of blood were incubated with antibody pairs for dual staining for 15 minutes at room temperature. Erythrocytes were lysed using FACS lysing solution (Becton Dickinson) and leucocytes were fixed with 0.5% formaldehyde. Cells were analysed on

the day of processing using a Becton Dickinson FACScan and Simulset software. This software analyses the lymphocyte gate and corrects the subsequent analysis for non-lymphocyte events.

Pretreatment values for lymphocyte subsets in the 36 patients with PMR/GCA were compared with controls. In addition, to see if patient selection was a critical factor, the patients with biopsy-proved GCA or the most severe symptoms of PMR, or both, were considered separately. This group of 13 was compared with controls and with the remaining group of 23 patients with less severe GCA/PMR.

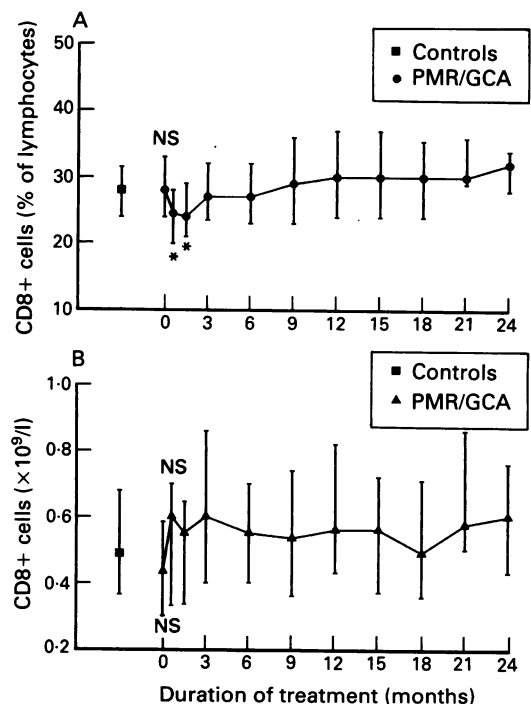
Statistical analysis consisted of the Mann-Whitney test to compare groups and paired analysis by the Wilcoxon test to examine within patient changes in T cell subsets.

Results

In all 36 patients with PMR/GCA before prednisolone treatment, lymphocytes, T cells, and T cell subtypes were all normal compared with controls (table 1).

After 10 days and three weeks of prednisolone treatment the absolute numbers of lymphocytes, T cells, and CD4+ cells had increased significantly, whereas CD8+ cells as a percentage of lymphocytes had decreased significantly (table 1).

The two year follow up of CD8+ cells is shown in the figure.



CD8+ T cells in controls and patients with polymyalgia rheumatic and giant cell arteritis (PMR/GCA) before and during treatment with prednisolone, after 10 days, six weeks, three months, and every three months thereafter (three week data points omitted for clarity; for three week data see table 1). During treatment venesection was carried out before the daily dose of prednisolone. Results expressed as percentage of lymphocytes (A) and as absolute numbers (B), medians plus interquartile range. * $p < 0.05$ and NS = not significant compared with controls.

Table 1 Lymphocytes and lymphocyte subtypes in controls and patients with polymyalgia rheumatica and giant cell arteritis (PMR/GCA) before treatment and after three weeks of treatment with prednisolone. Results expressed as medians, in absolute numbers, and as a percentage of lymphocytes

Parameter measured	Controls (n=36)	Patients with PMR/GCA before treatment (n=36)*	Patients with PMR/GCA after three weeks of treatment (n=34)†
Lymphocytes ($\times 10^9/l$)	1.67	1.47 (NS)	2.19 (p=0.005)
Total T cells ($\times 10^9/l$)	1.10	1.18 (NS)	1.59 (p=0.007)
(%)	68.5	71 (NS)	71.5 (NS)
CD4+ cells ($\times 10^9/l$)	0.75	0.78 (NS)	1.05 (p=0.002)
(%)	45	48 (NS)	50.5 (NS)
CD8+ cells ($\times 10^9/l$)	0.49	0.44 (NS)	0.49 (NS)
(%)	28	28 (NS)	25 (p=0.006)
CD4+:CD8+ ratio	1.59	1.74 (NS)	1.92 (p=0.006)
Activated T cells ($\times 10^9/l$)	0.12	0.11 (NS)	0.13 (NS)
(%)	6.5	8 (NS)	6 (NS)
NK cells ($\times 10^9/l$)	0.23	0.22 (NS)	0.17 (NS)
(%)	13	15 (NS)	8 (p=0.027)

*Values in parentheses are p values compared with controls.

†Values in parentheses are p values compared with pretreatment results.

Table 2 Lymphocytes and lymphocyte subtypes in the patients with most severe polymyalgia rheumatica and giant cell arteritis (PMR/GCA) compared with controls and with patients with less severe PMR/GCA. Results expressed as medians, in absolute numbers, and as a percentage of lymphocytes

Parameter measured	Controls (n=36)	Patients with severe PMR/GCA before treatment (biopsy proved GCA or severe PMR, or both) (n=13)*	Patients with less severe PMR/GCA before treatment (n=23)†
Lymphocytes ($\times 10^9/l$)	1.67	1.42 (p=0.048)	1.72 (NS)
Total T cells ($\times 10^9/l$)	1.10	1.05 (NS)	1.24 (NS)
(%)	68.5	71 (NS)	71 (NS)
CD4+ cells ($\times 10^9/l$)	0.75	0.71 (NS)	0.80 (NS)
(%)	45	50 (NS)	47 (NS)
CD8+ cells ($\times 10^9/l$)	0.49	0.36 (p=0.037)	0.48 (NS)
(%)	28	27 (NS)	30 (NS)
CD4+:CD8+ ratio	1.59	1.90 (NS)	1.58 (NS)
Activated T cells ($\times 10^9/l$)	0.12	0.14 (NS)	0.11 (NS)
(%)	6.5	9 (NS)	8 (NS)

*Values in parentheses are p values compared with controls.

†Values in parentheses are p values compared with patients with severe PMR/GCA.

We considered the group with severe PMR/GCA separately (table 2): this group had reduced lymphocyte and reduced CD8+ numbers before treatment compared with controls. CD8+ cells as a percentage of the lymphocytes were not reduced, however.

The subgroup of three patients with biopsy-proved GCA was too small for separate statistical analysis but their CD8+ counts were similar to the subgroup with severe PMR (as absolute numbers and as a percentage of lymphocytes).

Discussion

The results of this study do not confirm the conclusions of some previous studies that CD8+ cells are reduced in patients with GCA/PMR before treatment³⁻⁵ but agree with the studies of Andersson *et al*¹ and Banks *et al*² in finding no abnormality before treatment. Our previous finding that the percentage of CD8+ cells decreases significantly on treatment with prednisolone illustrates the importance of ensuring that initial samples are obtained before treatment is begun. In practice, this is

often difficult, as any delay in the treatment of PMR/GCA may lead to irreversible complications such as blindness.¹⁶ Hence recruiting untreated patients is not easy. We found that several patients seen rapidly for their first assessment and assumed to have received no corticosteroids had actually received the initial dose of prednisolone from their family doctor, and therefore were excluded from our study. This clinical history must be diligently sought and the magnitude of the effect of a single dose of corticosteroids appreciated.

Our work in normal volunteers^{14a} has shown that the effects of prednisolone on lymphocyte subsets occur in two phases. The initial effects (maximum at about seven hours after a 20 mg dose of enteric coated prednisolone) include a pronounced decrease in the absolute numbers of lymphocytes, total T cells, CD4+, and CD8+ cells. The effects of longer term prednisolone treatment are almost the opposite of the early effects—that is, at 72 hours the absolute numbers of total T cells, CD4+, and CD8+ cells are markedly increased, with a decrease in the percentage of CD8+ cells. These later effects begin to develop by 24 hours after the first dose of prednisolone. Before this study only acute lymphopenia (particularly of CD4+ cells) had been shown after a single dose of prednisolone in volunteers.^{17,18} The same effect has been assumed by some to occur in longer term treatment with corticosteroids, whereas our study in normal volunteers shows that the later changes contrast markedly with the acute effects. Those studies in PMR/GCA where patients were already receiving prednisolone at the time of the initial analysis of T cell subtypes^{6,7} cannot give us useful information about CD8+ numbers in PMR/GCA *per se*. In those studies reporting a reduced percentage of CD8+ cells in PMR/GCA before treatment,³⁻⁵ the inadvertent inclusion of patients after even a single dose of prednisolone might render these results similarly misleading. Multicentre studies may be particularly prone to this factor, in that it is more difficult at a distance to ensure that no treatment has been given before the initial blood sample.

During follow up of treated PMR/GCA, T cell subtypes will again be critically altered by the interval since the previous dose of prednisolone. In this study T cells during treatment were measured before the daily dose of prednisolone, and this is reflected in the long term increase of CD8+ numbers seen in the figure, though this did not reach statistical significance. Other studies have not referred to the timing of the blood samples. If blood had been taken a few hours after a prednisolone dose, then the absolute numbers of CD8+ cells would be lowered, and if blood had been taken about 24 hours after a dose of prednisolone then CD8+ cells as a percentage of lymphocytes would be lowered.^{14a} Hence studies reporting prolonged lowering of CD8+ cells in PMR/GCA may be showing the effects of prednisolone rather than the effects of PMR/GCA *per se*.^{4,6}

Some differences in the results of studies in patients with PMR/GCA might be due to patient selection. In our study, an increased ESR and CRP were not required (as these tests were also being investigated). This might have resulted in our patients being less florid than other series, though, in fact, 34 of 36 patients did have either an increased ESR or CRP. We have addressed this problem by separately analysing the data from the most severe cases of PMR together with the cases of GCA proved by biopsy. Although absolute numbers of CD8+ cells were lowered in this group, this was part of a slight overall lymphopenia and there was no selective depletion of CD8+ cells. This suggests that studies incorporating only the most severe cases of PMR/GCA may be more likely to show a lowered lymphocyte count and therefore a lowered absolute CD8+ count. CD8+ cells as a percentage of lymphocytes, however, do not seem to be influenced by disease severity. It is therefore unlikely that patient selection accounts for the difference between this study and other studies showing a reduced percentage of CD8+ cells.

The differing results from studies of CD8+ cells in patients with PMR/GCA raises doubts about whether CD8+ cells are actually reduced in these patients. We have illustrated design flaws which could distort data and lead to an erroneous conclusion that CD8+ cells are reduced in PMR/GCA. Published results may also be distorted in favour of an abnormality of CD8+ cells, in that negative studies showing no such abnormality are less likely to be published.¹⁹⁻²¹ We therefore conclude that the case that CD8+ cells are lowered in patients with PMR/GCA is not proved.

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- 1 Andersson R, Hansson G K, Soderstrom T, Jonsson R, Bengtsson B A, Nordborg E. HLA-DR expression in the vascular lesion and circulating T lymphocytes of patients with giant cell arteritis. *Clin Exp Immunol* 1988; 73: 82-7.
- 2 Banks P M, Cohen M D, Ginsburg W W, Hunder G G. Immunohistologic and cytochemical studies of temporal arteritis. *Arthritis Rheum* 1983; 26: 1201-7.
- 3 Elling P, Olsson A, Elling H. CD8+ T lymphocyte subset in giant cell arteritis and related disorders. *J Rheumatol* 1990; 17: 225-7.
- 4 Dasgupta B, Duke O, Timms A M, Pitzalis C, Panayi G S. Selective depletion and activation of CD8+ lymphocytes from peripheral blood of patients with polymyalgia rheumatica and giant cell arteritis. *Ann Rheum Dis* 1989; 48: 307-11.
- 5 Chelazzi G, Broggnini M. Abnormalities of peripheral blood T lymphocyte subsets in polymyalgia rheumatica. *Clin Exp Rheumatol* 1984; 2: 333-6.
- 6 Elling H, Elling P. Decreased level of suppressor/cytotoxic T cells (OKT8+) in polymyalgia rheumatica and temporal arteritis: relation to disease activity. *J Rheumatol* 1985; 12: 306-9.
- 7 Benlahrache C, Segond P, Auquier L, Bouvet J-P. Decrease of the OKT8 positive T cell subset in polymyalgia rheumatica. *Arthritis Rheum* 1983; 26: 1472-80.
- 8 Renzi P, Ginns L C. Analysis of T cell subsets in normal adults. Comparison of whole blood lysis technique to Ficoll-Hypaque separation by flow cytometry. *J Immunol Methods* 1987; 98: 53-6.
- 9 Ashmore L M, Shopp G M, Edwards B S. Lymphocyte subset analysis by flow cytometry. Comparison of three different staining techniques and effects of blood storage. *J Immunol Methods* 1989; 118: 209-15.
- 10 Bird A G. Monitoring of lymphocyte subpopulation changes in the assessment of HIV infection [editorial review]. *Genitourin Med* 1990; 66: 133-7.
- 11 Ritchie A W S, Oswald I, Micklem H S, Boyd J E, Elton R A, Jazwinska E. Circadian variation of lymphocyte subpopulations: a study with monoclonal antibodies. *BMJ* 1983; 286: 1773-5.
- 12 Levi F A, Canon C, Touitou Y, et al. Circadian rhythms in circulating T lymphocyte subtypes and plasma testosterone, total free cortisol in five healthy men. *Clin Exp Immunol* 1988; 71: 329-35.
- 13 Lighthart G J, Schuit H R E, Hijmans W. Subpopulations of mononuclear cells in ageing: expansion of the null cell compartment and decrease in the number of T and B cells in human blood. *Immunology* 1985; 55: 15-21.
- 14 Nagel J E, Chrest F J, Adler W H. Enumeration of T lymphocyte subsets by monoclonal antibodies in young and aged humans. *J Immunol* 1981; 127: 2086-8.
- 14a Pountain G D, Keogan M T, Hazleman B L, Brown D L. Effects of single dose compared with 3 days prednisolone treatment of healthy volunteers: contrasting effects on circulating lymphocyte subsets. *J Clin Pathol*. In press.
- 15 Jones J G, Hazleman B L. Prognosis and management of polymyalgia rheumatica. *Ann Rheum Dis* 1981; 40: 1-5.
- 16 Russell R W R. Giant-cell arteritis. A review of 35 cases. *Q J Med* 1959; 28: 471-89.
- 17 ten Berge R J M, Sauerwein H P, Yong S L, Schellekens P T A. Administration of prednisolone in vivo affects the ratio of OKT4/OKT8 and the LDH-isoenzyme pattern of human T lymphocytes. *Clin Immunol Immunopathol* 1984; 30: 91-103.
- 18 Tonnesen E, Christensen N J, Brinklov M M. Natural killer cell activity during cortisol and adrenaline infusion in healthy volunteers. *Eur J Clin Invest* 1987; 17: 497-503.
- 19 Easterbrook P J, Berlin J A, Gopalan R, Matthews D R. Publication bias in clinical research. *Lancet* 1991; 337: 867-72.
- 20 Dickersin K, Min Y I, Meinert C L. Factors influencing publication of research results. Follow-up of applications submitted to two institutional review boards. *JAMA* 1992; 267: 374-8.
- 21 Rennie D, Flanagan A. Publication bias. The triumph of hope over experience [editorial]. *JAMA* 1992; 267: 411-2.