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Decrease of regulatory T cells in patients with systemic lupus erythematosus

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Current evidence indicates that regulatory T cells (Tregs) actively suppress autoreactive lymphocytes that escape central tolerance.[1 2] Tregs are characterised by a high intensity CD25 constitutive expression[3] on the surface of CD4 T lymphocytes, which distinguishes them from the non-regulatory CD4+CD25+ T lymphocytes.[4] Because of their important role in the maintenance of tolerance, it has been suggested that Tregs are decreased in patients with systemic lupus erythematosus (SLE).[5] We have quantified Tregs in patients with SLE and controls and evaluated their association with disease activity and treatment.

Thirty three patients with SLE (26 women, 7 men) and 14 healthy volunteers matched for age and sex (11 women, 3 men) with a mean age of 39.9 and 36 years, respectively, were studied. All patients fulfilled at least four of the American College of Rheumatology 1982 revised criteria for the classification of SLE.[6] Disease activity at the time of blood sampling was assessed using the SLE Disease Activity Index (SLEDAI).[7] Seven patients had inactive disease (SLEDAI = 0), and the remaining 26 patients had a SLEDAI ranging from 1 to 12 (median 4).

Approval from our institutional ethics committees and informed consent from all patients and controls were obtained.

Tregs were identified in peripheral blood mononuclear cells from patients with SLE and controls by direct triple staining immunofluorescence with fluorescein isothiocyanate-antiCD4, phycoerythrin-antiCD25 (both from BD Biosciences), and tricolour-antiCD8 (Caltag) monoclonal antibodies. Data acquisition and analysis were performed with the CellQuest and Paint-A-Gate software, respectively, on a FACSort flow cytometer (BD Biosciences). Lymphocytes (10^4), gated according to forward and size scatter criteria, were analysed, and the percentage of CD4+ T cells with the brightest CD25 expression was calculated.

The Mann-Whitney U test and the Kruskal-Wallis test were used to compare two or more groups, respectively. Quantitative correlation between variables was assessed by Spearman's rank correlation.

No differences were found in the mean (SD) CD25 expression on CD4+ T lymphocytes between patients with SLE and controls (37.8 (11.9) *v* 31.3 (9.12), $p = 0.077$) and no correlation was detected between the SLEDAI score and the percentage of these cells in patients with SLE (data not shown). However, patients with SLE had a smaller percentage of CD4CD25bright T cells than controls, in comparison with both the total lymphocytes and the CD4+ T cells (1.29 (0.62) *v* 3.12 (1.41), $p<0.001$; 4.11 (1.93) *v*. 8.24 (2.93), $p<0.001$, respectively) (fig 1A). In addition, the SLEDAI score and the percentage of these cells were inversely correlated ($r_s = -0.611$, $p<0.001$) (fig 1B).

To assess whether the treatment the patients were receiving influenced the percentage of CD4CD25bright cells, patients were classified in five groups: (1) no treatment; (2) antimalarial drugs; (3) antimalarial drugs and low dose steroids ($\leqslant$15 mg/day); (4) immunosuppressive drugs (in combination with antimalarial drugs and/or low dose steroids); (5) antimalarial drugs and high dose steroids (prednisone 30 mg/day). Treatment groups with fewer than three patients were excluded from the statistical analysis. All groups, including patients not receiving treatment, had a lower percentage of CD4CD25bright T cells than controls ($p<0.001$) (fig 1C); however, no differences were found among the groups of patients ($p = 0.11$).

To date there is no consensus about the role of Tregs in the pathogenesis of SLE. Some authors have found that Tregs are decreased in patients with SLE but that they did not correlate with disease activity.[8] However, Crispin *et al* showed that only patients with active SLE had a decreased percentage of CD4+CD25bright T cells.[9]

Our results suggest that patients with SLE have a lower percentage of CD4CD25bright T cells, which decreases as the disease activity increases and which is independent of the treatments they are receiving at the time of the study. These observations support the hypothesis that Tregs might be involved in the pathogenesis of SLE.

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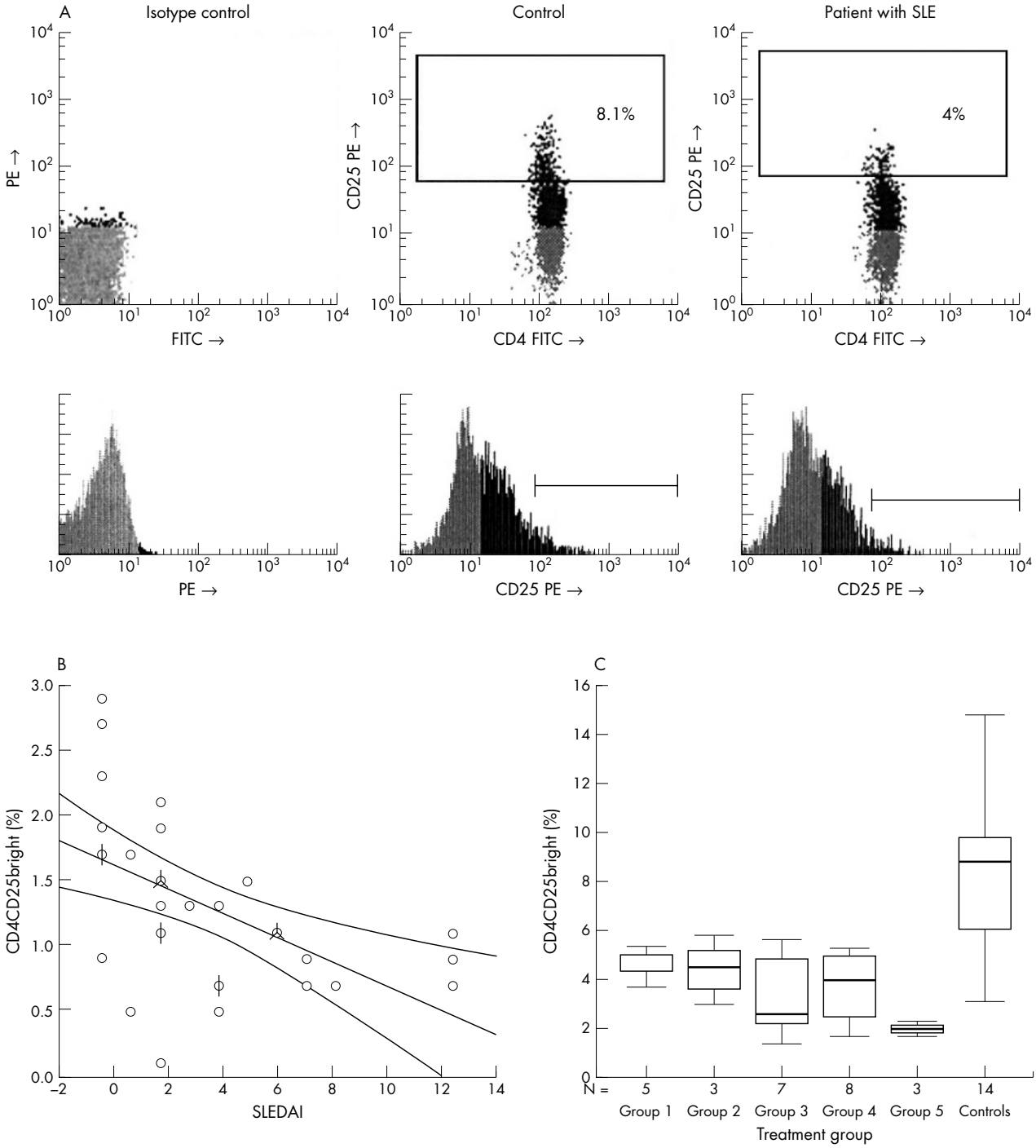

Figure 1 (A) Flow cytometry dot plots (upper) and histograms (lower) showing the isotype control expression and the CD25 expression on gated CD4+ T lymphocytes from one representative control and one patient with SLE. The percentage of CD4CD25bright cells is indicated by an open rectangle. (B) Correlation between the SLEDAI score and the percentage of CD4CD25bright T cells, calculated from the total lymphocytes ($r_s = -0.611$, $p<0.001$; Spearman's rank correlation). (C) Box plots representing the percentage of CD4CD25bright cells within the CD4+ T lymphocytes in patients with SLE grouped according to the treatment they were receiving, and the controls. The bar within the box represents the median value. The outlines of the boxes show the 25th and the 75th centiles, while the bars outside the boxes represent the 10th and 90th centiles. $p<0.001$ among all groups, and $p=0.11$ among treatment groups (both by the Kruskal-Wallis test).

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