Growth of human B cell colonies from peripheral blood of patients with systemic lupus erythematosus

ANNE C FAY,¹ SUSAN PRICE,¹ A TRUDGETT,¹ J D McCREA,²
MARGARET HAIRE,¹ M W J BOYD,² AND T A McNEILL¹

From the ¹Department of Microbiology and Immunobiology, Queen's University Belfast, Belfast; and the ²Department of Rheumatology, Musgrave Park Hospital, Belfast

SUMMARY Human B cell colonies were grown from peripheral blood of 12 patients with systemic lupus erythematosus (SLE) and from 12 healthy control subjects. The SLE group showed a large increase ($p < 0.001$) in the number of colony forming cells (CFC) present in peripheral blood as compared with controls. The CFC were of the pre-B cell type. There was also a loss of OKT8⁺ cell inhibition of B cell colony growth in the SLE group compared with control subjects.

B cell hyperactivity is a prominent feature of systemic lupus erythematosus (SLE),¹ with active SLE characterised by hypergammaglobulinaemia, increased spontaneously activated B cells,² and increased immunoglobulin (Ig) secreting cells.³ Generalised T suppressor cell defects have frequently been suggested as a cause of this B cell hyper-reactivity⁴ but have not been consistently demonstrated.^{5,6} Kumagi and his coworkers (1982),⁷ with a B cell colony growth system which employed T cell derived supernatants as a source of B cell growth factors, showed an increased number of B cell colony forming cells to be present in peripheral blood of patients with SLE as compared with normal controls. Although the nature of the colony forming cells (CFC) in their system was not demonstrated, these results would suggest that a B cell lineage stem cell defect may exist in human SLE.

We have previously described a B cell colony growth system which uses conditioned medium (CM) from a human plasmacytoma cell line (HMy2) as a source of B cell growth factors.⁸ The majority of the CFC in this system are relatively immature, sIg⁻, pre-B cells, thus enabling us to study B cell activation at an earlier stage than Kumagi's group. The growth factors present in the HMy2 CM differ from previously described T cell derived B cell growth factors and may be a human analogue of interleukin-3.⁹

In the present study we have used this system to analyse B cell colony growth from pre-B cells in SLE patients in the absence of human T cells or their products, and we have also been able to investigate the ability of autologous OKT8⁺ (suppressor/cytotoxic) T cells to inhibit B cell colony growth in both SLE patients and in normal subjects.

Patients and methods

PATIENTS WITH SLE

Blood samples were obtained from 12 female patients with SLE whose diagnoses were made with the 1982 revised criteria of the American Rheumatism Association.¹⁰ Their ages ranged from 21 to 67 years with a mean of 44 years, and the duration of their diseases varied between two and 37 years. All were taking non-steroidal anti-inflammatory drugs or analgesics at the time of the study but not corticosteroid or cytostatic drugs.

DISEASE ACTIVITY

This was assessed with the scoring system reported by Morimoto *et al.* in 1982.¹¹ This gave a maximum possible score of 49 points.

CONTROL POPULATION

Twelve healthy, female subjects whose ages ranged from 19 to 23 years comprised the control population.

HUMAN B CELL COLONY CULTURE

The panning techniques used in the preparation of cell populations for colony culture and details of

their subsequent culture have been described elsewhere.⁸ The B cell nature of the resulting colonies was checked after seven days' incubation by removal of individual colonies and immunofluorescent staining as described below.

ENUMERATION OF B AND T LYMPHOCYTES
This was carried out by indirect immunofluorescence using the OKT3, OKT4, and OKT8 mouse antihuman monoclonal antibodies (Ortho, UK) or a mouse antihuman immunoglobulin antibody as appropriate. A goat fluorescein conjugated anti-mouse second antibody (Ortho) was then used. Cell preparations were prepared according to the manufacturer's instructions (Ortho, 1983) and examined with a fluorescence microscope.

OKT8 T CELL SUBSET ENRICHED POPULATION
This was prepared from the cell population which was not adherent to anti-immunoglobulin coated plates. Only the cells in the original 2.5 ml volume applied were used, subsequent washings being discarded. This cell population contained on average 90–95% T lymphocytes, as measured by E rosetting. After incubation in monoclonal antibody (Ortho) and dilution in phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (titre 1/1000 to 1500, 500 µl for 3×10⁷ cells) for 30 minutes at 4°C the cells were washed twice in cold PBS, resuspended in PBS supplemented with 5% fetal bovine serum (FBS), and added to a plate previously precoated with 10 µl of affinity purified goat antimouse Ig (Northeast Biomedical Laboratories, UK). The non-adherent cells were removed and the adherent cells eluted by incubation at 37°C overnight in RPMI 1640 supplemented with 15%

mouse serum. Viability was assessed by trypan blue dye exclusion. This procedure gave better than 90% of cells bearing the OKT8 surface marker.

COCULTURE EXPERIMENTS
In some experiments OKT8⁺ cells were prepared as described above. A mixture of the appropriate numbers of the B enriched population (2×10⁵ cells per dish) and a known number of OKT8⁺ cells (varying between 1×10⁴ and 1×10⁶ cells) was added to each dish. The cultures were incubated and the colonies counted.

Results

GENERAL LABORATORY RESULTS IN SLE PATIENTS
All 12 SLE patients showed normal levels of serum IgG (Table 1). Three of the 12 patients had increased levels of antibodies to double stranded deoxyribose nucleic acid (dsDNA) present in their sera, four patients had reduced complement component C4 levels in their sera, and 10 patients had increased levels of circulating immune complexes in their sera.

B CELLS, B CELL COLONY FORMING CELLS AND T CELL SUBSETS IN PERIPHERAL BLOOD FROM SLE PATIENTS
These results are presented in Table 2. There was no significant difference in B cell numbers between the SLE patients and normal controls. There was, however, a statistically significant (p<0.001) increase in the number of B cell colonies grown from the peripheral blood of SLE patients as compared with normal subjects. The cells in the colonies were on average 91% cytoplasmic Ig⁺ with, on average

Table 1 General laboratory characteristics of SLE patients

Patient No	ANA* (titre)	dsDNA (units/ml)	C3 (g/l)	C4 (g/l)	IC-Raji* (µg AHG equiv)	ENA*	IgG (g/l)
1	20	12	0.94	0.27	250	Ro	6.4
2	320	7	1.20	0.34	1000	RoLa	9.7
3	0	0	0.98	0.34	79	—	6.1
4	40	2	0.74	0.29	50	—	8.7
5	20	4	1.10	0.44	70	RoLa	13.2
6	80	0	0.84	0.26	79	RoLa	9.5
7	640	45	0.82	0.02	1412	RoLa	10.6
8	160	31	0.98	0.41	1258	RoLa	9.4
9	320	73	0.67	0.12	158	SmRNP	11.9
10	160	7	0.94	0.12	49	RoLa	12.3
11	20	19	1.10	0.23	100	—	8.2
12	320	10	0.82	0.13	20	RoLa	9.1

*ANA=antinuclear antibody; IC=immune complex; ENA=extractable nuclear antigen.
Normal ranges: C3 0.5–1.2 g/l, C4 0.2–0.5 g/l, IgG 6.0–15.9 g/l, dsDNA 0–25 units/ml, IC-Raji 0–49 µg aggregated human globulin equivalent.

Table 2 Comparison of B cells, B cell colony forming cells, and T cell subsets in peripheral blood from SLE patients and normal controls

	Control	SLE
B cells (sig+ve)*	0.09±0.04†	0.11±0.04
B CFC	417±24	1290±440
OKT3+	1.09±0.38	0.70±0.31
OKT4+	0.77±0.28	0.45±0.26
OKT8+	0.36±0.16	0.29±0.16

*B cell and T cell numbers are expressed as cells/ 1×10^{-9} . B CFC numbers are expressed as per 10^5 B cells.

†Values are mean ±SD.

Fig. 1 Human B cell colony growth and disease activity in SLE patients. Normal limits for colony growth are indicated by cross hatching. All cultures were set up at 2×10^5 cells/ml (2×10^6 /l). Results of 12 individual patients are shown.

8% E rosette⁺ cells also present. A statistically significant difference in the number of OKT3⁺ (pan-T) cells and OKT4⁺ (helper/inducer) cells present in the two groups was also noted ($p < 0.01$). There was no significant difference in OKT8⁺ (suppressor/cytotoxic) cell numbers between the two groups.

RELATIONSHIP BETWEEN DISEASE ACTIVITY AND B CELL COLONY NUMBER

These results are shown in Fig. 1. There was a statistically significant ($p < 0.001$), positive correlation ($r = 0.883$) between B cell colony number in SLE and clinical score.

NATURE OF THE CFC

The results given in Table 3 show that the CFC bear the Leu 12 epitope and therefore belong to the B cell lineage. However, the majority of the CFC lack surface Ig, with only a small minority (c 20%) possessing sIgM. This would suggest that the majority of the CFC are relatively immature pre-B cells.

COCULTURE EXPERIMENTS

Fig. 2 shows the results of experiments in which autologous OKT8⁺ cells were cocultured with the B enriched cell population (see 'Patients and methods'). Complete inhibition of colony growth occurred in the control group at a T:B cell ratio of 0.35:1, whereas no inhibition of colony growth occurred in the SLE group even at T:B cell ratios of 2.5:1. The cell composition of the colonies in these coculture experiments was on average 91% cIg⁺ at seven days.

Neither coculture of SLE derived B cells with T cells from normal controls nor SLE derived T cells with B cells from normal controls gave rise to colony growth.

Discussion

We have observed a large increase in the number of B cell colony forming cells present in the peripheral blood of patients with SLE when compared with

Table 3 Depletion of cells by surface antigen

Antigen bearing cell removed	Colonies formed from residual cells (% of control)
Leu 12	4.1
IgM	82.4
IgG	96.8
IgA	92.6
IgD	103.2
IgE	91.7

Fig. 2 Coculture experiments with B enriched cell population and OKT8⁺ cell population. Results are mean $\pm$ SD of 12 experiments. SLE patients (●); controls (○).

normal controls, and this increase correlated highly with the clinical activity of the patients' disease. The majority of colony forming cells in the peripheral blood lacked surface immunoglobulin but expressed the Leu 12 surface antigen, which is found on cells of the B cell lineage but not on macrophages or activated T cells.¹² During B cell ontogeny the Leu 12 antigen is expressed at a similar stage of differentiation as the HLA-DR surface antigen and before the appearance of surface immunoglobulin (Crockard, personal communication). Therefore the majority of the colony forming cells present in the peripheral blood of SLE patients, as in normal subjects,⁸ are of the pre-B cell family.

Kumagi and his coworkers⁷ first reported the growth of human B cell colonies from peripheral blood of patients with SLE in 1982. They found that increased numbers of colonies could be cultured from blood from SLE patients as compared with normal controls. In their colony assay system lipopolysaccharide was used as a non-specific mitogen and T cell conditioned medium as a source of B cell growth factors. The nature of the colony forming cell was not made clear; however, other workers using very similar systems have shown that T cell derived growth factors act on relatively mature sIg⁺ B cells.^{13 14}

Interestingly, although the number of CFC (pre-B cells) present in the peripheral blood was markedly increased in our SLE patients compared with normal controls, the number of mature B cells detected by sIg production did not differ significantly between the two groups. Other workers have found reduced absolute numbers but increased percentages of circulating B cells in peripheral blood of patients with active SLE.¹⁵

B cell hyper-reactivity has also been shown in human SLE by a reverse haemolytic plaque assay. These workers found that the spontaneous plaque forming cell response in patients with SLE was increased and that the levels correlated positively with clinically defined disease activity and with the degree of renal disease as assessed by renal biopsy. However, there are a number of reports which have shown that pokeweed mitogen fails to stimulate peripheral blood mononuclear cells from SLE patients to differentiate into immunoglobulin secreting plaque forming cells.^{16 17} Pokeweed mitogen is absolutely dependent on the presence of human T cells for its mitogenic effect on B cells,¹⁸ so these results may reflect a failure of T cell help as well as of B cell response. As pokeweed mitogen acts on a relatively mature sIg⁺ B cell,¹⁹ it would appear that in human SLE, though there is an

increase in the number of immature B cells in the peripheral blood capable of growth and differentiation in a colony assay system and an increase in the amount of total immunoglobulin produced, there is no increase in the number of mature slg^+ B cells present in the peripheral blood, and these cells fail to respond to non-specific mitogens such as pokeweed.

Our results for T cell subset analysis are in keeping with those reported by Frazer and Mackay²⁰ who studied 10 patients with SLE with varying disease activities. They found significant reductions in mean T cell count and T helper cell count in the SLE group as compared with controls. They did not find a significant difference in suppressor T cell numbers between the two groups. Some of their SLE group patients were taking immunosuppressive therapy at the time of study. Stohl²¹ has reported that the OKT4 epitope may be deficient on the helper cells of patients with SLE, though the Leu 3a epitope is preserved. It is possible that this phenomenon was responsible for the reduction in OKT4⁺ cell numbers which we found in our SLE patients, as all our experiments were carried out using the OKT (Ortho) series of monoclonal antibodies.

A significant decrease in the numbers of OKT8⁺ (suppressor/cytotoxic) cells in peripheral blood of patients with SLE as compared with normal controls has not been found. However, there was a marked difference in OKT8⁺ cell function between the two groups, with failure of inhibition of B cell colony growth even at very high T:B cell ratios in the SLE population.

The failure of SLE OKT8⁺ cells to inhibit colony growth in our experiments may be due either to failure of the T cells to initiate suppression or to failure of the B cells to respond. Attempts to distinguish between these two possible explanations with coculture experiments were unsuccessful, as there was no B cell colony growth in the presence of allogeneic T cells. This is similar to the effect seen when allogeneic T cells, not matched at the HLA-A or B locus, are added to Epstein-Barr virus stimulated B cells.²²

There are three possible mechanisms by which normal OKT8⁺ cells could inhibit B cell colony growth. It may be a cytotoxic effect, a suppressor effect, or the T cells may preferentially absorb the growth factors present in the HMy2 CM. It is not possible at this stage to distinguish between these potential mechanisms. Of interest is the observation that no inhibition of colony growth occurred in the normal population, even at very high T:B cell ratios, if the OKT8⁺ T cells were added after three days of colony culture rather than at the beginning of the

culture period (unpublished results), suggesting that they act on a surface Ig^- B cell.⁸ This would be in keeping with the results of Calderon and Thomas²³ who showed cyclic changes in the susceptibility of memory B cells to T cell help and T cell suppression.

There have been conflicting reports about the presence of a suppressor T cell defect in SLE.²⁴⁻²⁵ In general, suppressor T cell defects have been most often shown in patients with active disease⁶ but it is not clear whether such defects are caused by the disease process or are responsible for it. It has been shown that SLE patients produce anti-T cell antibodies and that these antibodies can have specificities for the suppressor T cell subset,²⁶ pointing to the T cell defects¹ being consequences of the disease process.

To conclude, we have shown greatly increased numbers of colony forming B cells of pre-B cell type in SLE, and this increase correlated with clinical disease activity. A functional T suppressor cell defect was also demonstrated.

References

- 1 Steinberg A D (moderator). Systemic lupus erythematosus: insights from animal models. *Ann Intern Med* 1984; **100**: 714-27.
- 2 Balow J E, Tsokos G C. T and B lymphocyte function in patients with systemic lupus nephritis: correlation with renal pathology. *Clin Nephrol* 1984; **21**: 93-7.
- 3 Blaes R M, Grayson J, Steinberg A D. Elevated immunoglobulin secreting cells in the blood of patients with active systemic lupus erythematosus: correlation of laboratory and clinical assessment of disease activity. *Am J Med* 1980; **69**: 345-50.
- 4 Mountz J D, Steinberg A D. Recent advances in SLE. *Clin Immunol Newsletter* 1984; **5**: 33-7.
- 5 Sakane T, Steinberg A D, Green I. Studies of immune functions of patients with systemic lupus erythematosus. V. T cell suppressor function and autologous MLR during active and inactive phases of disease. *Arthritis Rheum* 1980; **23**: 225-31.
- 6 Steinberg A D. Immune regulatory abnormalities in systemic lupus erythematosus. In: Cummings N, Michael A, Wilson C, eds. *Immune mechanisms of renal disease*. New York: Plenum, 1983: 520-39.
- 7 Kumagi S, Sredni B, House S, Steinberg A D, Green I. Defective regulation of B lymphocyte colony formation in patients with systemic erythematosus. *J Immunol* 1982; **128**: 258-62.
- 8 Fay A C, Trudgett A, Thompson J M, McNeill T A. Human B cell colony growth from pre-B cells in vitro. *Clin Exp Immunol* 1985; **59**: 629-36.
- 9 Trudgett A, Fay A C, Thompson J M, McNeill T A. Biochemical and physicochemical characteristics of a pre-B cell stimulating factor produced by the plasmacytoma cell line LICR LON HMy2. *Clin Exp Immunol* 1985; **59**: 637-43.
- 10 Tan E M, Cohen A S, Fries J T, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**: 1271-7.
- 11 Morimoto C, Sano H, Abe T, Homma M, Steinberg A D. Correlation between the clinical activity of systemic lupus erythematosus and the amounts of DNA in DNA/anti-DNA antibody immune complexes. *J Immunol* 1982; **139**: 1960-6.
- 12 Meeker T C, Miller R, Link M, Bindle J, Warnke R, Levy R. A novel B lymphocyte antigen defined by a monoclonal antibody. *Hybridoma* (in press).

- 13 Whisler R, Newhouse Y. Characteristics and differential T cell dependency of human B cell colony precursors. *Cell Immunol* 1982; **67**: 346–54.
- 14 Kuritani T, Kishimoto T, Muraguchi A, Yamamura Y. In vitro immune response of human peripheral lymphocytes VI. *J Immunol* 1980; **125**: 2633–7.
- 15 Morimoto C, Reinherz E L, Nadler L M, Distaso J A, Steinberg A D, Schlossman S F. Comparison of T and B cell markers in patients with Sjögren's syndrome and systemic lupus erythematosus. *Clin Immunol Immunopathol* 1982; **22**: 270–8.
- 16 Ginsberg W W, Finkleman F D, Lipsky P E. Circulating and pokeweed mitogen induced immunoglobulin-secreting cells in systemic lupus erythematosus. *Clin Exp Immunol* 1979; **35**: 76–88.
- 17 Tsokos G C, Balow J E. Spontaneous and pokeweed mitogen induced plaque forming cells in systemic lupus erythematosus. *Clin Immunol Immunopathol* 1981; **21**: 172–83.
- 18 Waldmann T A, Broder S. Polyclonal B cell activators in the study of the regulation of immunoglobulin synthesis in the human system. *Adv Immunol* 1982; **32**: 1–63.
- 19 Ault K A, Towle M. Human B lymphocyte subsets. I. IgG-bearing B cell response to pokeweed mitogen. *J Exp Med* 1981; **153**: 339–51.
- 20 Frazer I H, Mackay I R. T Lymphocyte subpopulations defined by two sets of monoclonal antibodies in chronic active hepatitis and systemic lupus erythematosus. *Clin Exp Immunol* 1982; **50**: 107–14.
- 21 Stohl W. Deficient OKT4 epitope on helper T cells in a patient with AIDS. *New Engl J Med* 1984; **310**: 1531.
- 22 Blaese R M, Tosato G. In: Fauci A S, Ballieux R, eds. *Human B cell lymphocyte function: activation and immunoregulation*. New York: Raven, 1982.
- 23 Calderon R A, Thomas D B. In vivo cyclic change in B lymphocyte susceptibility to T cell control. *Nature* 1980; **285**: 662–4.
- 24 Kammer G M, Birch R E, Polmar S H. Impaired immunoregulation in systemic lupus erythematosus: defective adenosine-induced suppressor T lymphocyte generation. *J Immunol* 1983; **130**: 1706–11.
- 25 Tsokos G C, Balow J E. Phenotypes of T lymphocytes in systemic lupus erythematosus: decreased cytotoxic/suppressor subpopulation is associated with deficient allogeneic cytotoxic responses rather than with concanavalin A-induced suppressor cells. *Clin Immunol Immunopathol* 1983; **26**: 267–76.
- 26 Morimoto C, Reinherz E L, Distaso J A, Steinberg A D, Schlossman S F. Relationship between systemic lupus erythematosus T cell subsets, anti-T cell antibodies and T cell functions. *J Clin Invest* 1984; **73**: 689–700.