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Specific allogeneic help by T lymphocytes from patients with systemic lupus erythematosus

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SUMMARY

Unfractionated mononuclear cells from patients with systemic lupus erythematosus (SLE) immunized with influenza vaccines do not produce a secondary *in vitro* anti-influenza antibody response when challenged with virus antigen. Irradiated T lymphocytes from normal, disease control and from SLE donors whether vaccinated or not, help allogeneic normal non-T cells to produce specific anti-influenza antibody *in vitro*. Irradiated normal T cells, however, do not help allogeneic non-T cells from SLE donors. Non-irradiated T cells from 40% of the SLE patients, irrespective of whether or not they had been vaccinated, also provide specific help for MLC incompatible normal non-T cells in the influenza antibody response. This non-restricted interaction was not seen using non-irradiated T cells from any normal or disease control donor. No anti-DNA antibodies were produced in virus stimulated cultures of non-irradiated or irradiated SLE T cells with allogeneic normal non-T cells.

Keywords specific allogeneic help T lymphocytes systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized both by an excess of circulating immunoglobulin (Jasin & Ziff, 1979) and by the production of autoantibodies to self-antigens normally immunologically quiescent. Autoantigens recognized by antibodies in the sera of SLE patients include nuclear proteins (Scopelitis, Biundo & Alspaugh, 1980), nucleic acids (Vaughan & Chihara, 1973), and T lymphocyte surface molecules (Sakane *et al.*, 1979). Both the increased total immunoglobulin production and the production of specific autoantibodies, have been demonstrated *in vitro* using peripheral blood lymphocytes (PBL) from patients with SLE.

The production of antibodies to self and non-self antigens by B lymphocytes is normally influenced by T lymphocytes, acting to increase (help), or reduce (suppress) antibody production, respectively. These interactions between T and non-T cells normally require that the participating T and non-T cell fractions be histocompatible. In normal donors, this histocompatibility requirement can be overcome if the T cells are irradiated, or if cells of suppressor/cytotoxic phenotype are removed from the T cell population (Callard & Smith, 1981).

There is some evidence that antibody responses to specific antigens by PBL from patients with SLE may be abnormal *in vitro*. For example, following immunization with influenza vaccine, PBL from a majority of SLE patients fail to produce specific anti-influenza antibodies after *in vitro* challenge with influenza virus; in contrast, PBL from normal volunteers immunized in parallel

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produce high levels of specific antibody. The increases in serum haemagglutination inhibiting (HI) antibody titre are, however, similar in the SLE and normal groups (Mitchell *et al.*, 1982).

Accordingly, we have studied the ability of irradiated and non-irradiated T lymphocytes from SLE patients to help specific anti-influenza antibody production by allogeneic normal B lymphocytes. Irradiated T cells from both normal donors and SLE patients provided specific allogeneic help, irrespective of whether the donors had been vaccinated or not. Unlike non-irradiated T cells from any normal donor studied, however, non-irradiated T cells from approximately 40% of the SLE patients helped MLC incompatible normal non-T cells to make specific antibody, again irrespective of the vaccination status of the donor. This incompatible T–non-T cell collaboration was not associated with the production *in vitro* of anti-DNA antibodies.

MATERIALS AND METHODS

*Patients.*Twenty-seven patients were studied. All SLE patients studies fulfilled four or more of the revised criteria for the classification of SLE established by the ARA (Tan *et al.*, 1982). Disease activity was assessed by the UCH/Middlesex Hospitals protocol (Morrow *et al.*, 1981). Eighteen of the 27 patients studied were receiving up to 40 mg per day of prednisolone and three were taking up to 200 mg per day of azathioprine. Other clinical and pathological features of the patients are shown in Table 2.

A total of 19 disease control patients (13 female and six male) were studied in parallel. Four patients were receiving prednisolone (maximum dosage 20 mg daily), and one azathioprine at the time of study. Seven patients had seropositive rheumatoid arthritis and three a cerebrovascular accident. The diagnoses in the remaining nine were: diabetes, cholecystitis, myocardial infarction, prostatic hypertrophy with urinary tract infection, asthma, multiple sclerosis, Wegener's granulomatosis, hepatic cirrhosis and low back pain.

All SLE and disease control patients had given informed consent for these studies. Normal control donors were healthy laboratory staff.

Lymphocyte preparation and fractionation. Lymphocytes from normal and patient donors were prepared and fractionated into E rosette positive (ER^+) and ER^- cells using S-2-aminoethylthiouronium bromide sensitized sheep erythrocytes as described previously (Callard & Smith, 1981).

Lymphocyte characterization. Unfractionated lymphocytes from patients and normal control donors were characterized by indirect immunofluorescence using the monoclonal antibodies (MoAb) UCHT1 (pan-T) (Beverley & Callard, 1981), UCHT4 (suppressor/cytotoxic) (Beverley,

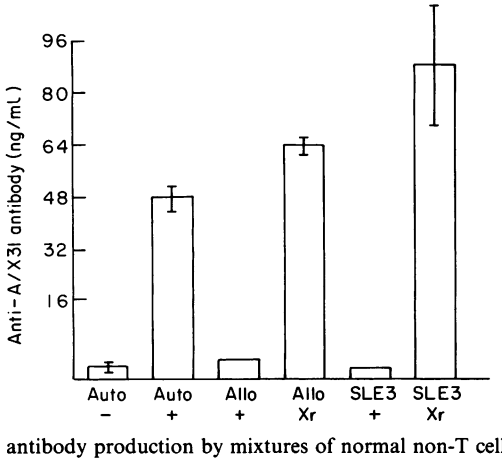

Fig. 1. Specific anti-A/X31 antibody production by mixtures of normal non-T cells with non-irradiated and irradiated (XR) autologous, allogeneic normal (Allo), and allogeneic SLE (SLE3) T cells with (+), or without (−) influenza virus (strain A/X31) antigen.

1982), both kindly provided by Dr P. Beverley, and Leu 3a (Ledbetter, *et al.*, 1981) (helper/inducer; Beckton Dickinson).

Antibody assays. Anti-influenza and anti-DNA antibodies were assayed by previously published ELISA methods (Callard & Smith, 1981; Shoenfeld *et al.*, 1982).

Assay for total IgG. The total IgG concentration of culture supernatants was determined by a competition assay. Round bottomed microtitre wells were incubated with 75 μl of BSA in PBS for 1 h at 37°C to block non-specific binding sites. After washing 60 μl of test supernatant and 15 μl of goat anti-human IgG–alkaline phosphatase conjugates (GaHIg-AP) was added to each well and incubated for 1 h. Separately, flat bottomed microtitre plates were coated with purified human immunoglobulin (75 μl at 0·1 mg/ml) for 1 h, the plates washed, and non-specific binding sites blocked with 1% BSA in PBS. After washing, 60 μl of the supernatant GaHIg-AP mixture was added to each well to detect binding of residual GaHIg-AP to the immunoglobulin bound to the flat bottomed plate. After incubation for 1 h at 37°C and further washing, *p*-nitrophenyl phosphate substrate was added to each well. Subsequent steps were performed in the same way as described in the assays for specific antibodies.

Mixed lymphocyte cultures. Mixed lymphocyte cultures were set up and pulsed exactly as described by Fitzharris & Knight (1981).

RESULTS

Antibody response in vitro *following influenza immunization*

Nine patients with SLE and four normal volunteers were immunized with influenza virus haemagglutinin. In agreement with the earlier studies of Mitchell *et al.* (1982) lymphocytes from all four normal donors produced increased amounts of specific antibody *in vitro* after immunization. In contrast, no specific antibody was produced by cells from any of the nine immunized SLE patients after virus challenge *in vitro* (data not shown). All immunized donors, however, both normals and those with SLE, showed post-immunization increases in serum antibody titre.

Cell mixing studies

Irradiated T cells. The *in vitro* antibody response to influenza virus requires HLA histocompatibility between participating ER^+ and ER^- fractions. Low dose irradiation (1,000 rad) abolishes this histocompatibility requirement and allows the production of specific antibody by allogeneic mixtures of T and non-T cells. Fig. 1 shows a representative experiment comparing the ability of X-irradiated disease control and SLE T cells to help allogeneic normal non-T cells to produce specific antibody when challenged with virus *in vitro*. In the experiment shown, non-irradiated

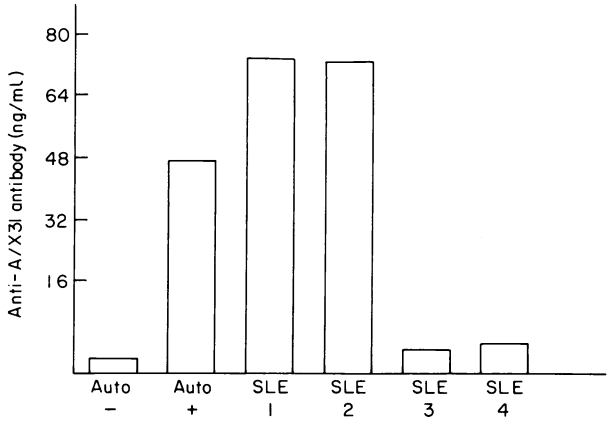

Fig. 2. Specific anti-A/X31 antibody production by mixtures of normal non-T cells with non-irradiated T cells from autologous (Auto) and allogeneic SLE (SLE1–4) donors, with (+) and without (−) A/X31 viral antigen.

disease control and SLE T cells do not provide help. When the disease control and SLE T cells are irradiated, however, allogeneic help results in the production of specific anti-influenza antibody. Irradiated T cells from 23 of 24 patients, from 19 of 19 disease control patients and from all 27 normal donors provide specific allogeneic help in this way.

We have not seen the *in vitro* production of specific antibody by SLE non-T cells cultured with either autologous, or irradiated and non-irradiated allogeneic normal T cells.

Non-irradiated T cells. Non-irradiated T cells from a proportion of SLE patients can also help allogeneic non-T cells from a normal influenza responding donor to produce specific antibody. In the experiment shown in Fig. 2, specific antibody concentration was measured in supernatants of cultures containing normal non-T cells and either autologous T cells or non-irradiated T cells from four SLE patients. Specific antibody was produced in two of the allogeneic combinations in amounts comparable with those produced by the autologous combination. Cultures containing irradiated T cells from these same two SLE patients and the same normal non-T cells also contained similar amounts of specific antibody (data not shown).

From our current experience, non-irradiated T cells from a total of 11 of 27 non-immunized SLE patients provided specific allogeneic help. Of the nine immunized patients, non-irradiated T cells from three helped allogeneic non-T cells both before and after immunization; cells from a fourth male patient only provided allogeneic help after immunization. Allogeneic help was seen on five occasions over an 8 month period by non-irradiated T cells from one non-immunized patient. The T cells from both this and one other patient helped non-T cells from more than one allogeneic normal donor, the normal donors used themselves being MLC incompatible. We have not observed specific allogeneic help by non-irradiated T cells from any of the 19 disease control or 27 normal donors tested.

The clinical and pathological features of patients whose T cells provide allogeneic help after irradiation only ('non-responders') or both without and with irradiation ('responders') are compared in Table 1. There are no significant differences between the two groups. In particular, individual patients in both responder and non-responder categories were not receiving steroids or immunosuppressants.

Table 1. Clinical and pathological characteristics of SLE patients whose T cells provide specific allogeneic help without as well as with irradiation ('responders'), and those whose T cells help only after irradiation ('non-responders').

	Non-responders ($n=16$)	Responders ($n=11$)
Clinical status		
Inactive	5	7
Mod. active	8	4
Severely active	3	0
Years since diagnosis (mean)	3·5	3
No. on oral steroids	11	7
Mean dose prednisolone (mg/day)	6·8 (0–30)	11·6 (0–40)
Total PBL $\times 10^{-6}$/ml	1·43	1·64
Circulating IC (normal 100)	124·7	113·9
PEG (mean μg/ml)		
No. with elevated CIC (%)	7 (43·7)	3 (27·2)
Mean C3 (normal 860–1,860 mg/l)	1,280	1,436
No. with low C3	4	0
No. with high C3	2	1
ESR (normal 10 mm/h)	46·7 ± 31·3	59·6 ± 25·0

* Normal 100.

Mixed lymphocyte reaction

To confirm that those SLE patients whose non-irradiated T cells provided specific allogeneic help were truly allogeneic with the normal non-T cells used in each experiment, parallel MLC were established. Fig. 3 shows specific antibody production, and the MLC response, when non-irradiated T cells from one normal, and two SLE donors were cultured with allogeneic normal and non-T cells with and without virus, respectively. A significant, and roughly comparable, allogeneic MLC response is seen with all three allogeneic cultures. However, only the non-irradiated T cells from patient SLE 1 provided specific allogeneic help in the presence of virus. Similarly, MLC incompatibility between the SLE and normal donors was demonstrated in all other examples of specific allogeneic help.

T cell phenotype

Lymphocytes from nine SLE patients were analysed using T cell set and subset specific MoAb at the same time as they were tested for specific allogeneic help. T cells from five of these patients provided allogeneic help both with and without irradiation ('responders'); T cells from the remaining four patients only helped allogeneic cells after irradiation ('non-responders').

The mean percentage of T cells in both SLE patient groups was not significantly different from normal, although in two patients (one responder and one non-responder) T cells were only 50% of total lymphocytes. Similarly, the mean ratios of T cells of helper/inducer:suppressor/cytotoxic phenotype ($1{\cdot}75 \pm 1{\cdot}12$ in responders and $1{\cdot}96 \pm 1{\cdot}10$ in non-responders) were neither significantly different from each other, nor from normal ($1{\cdot}72 \pm 0{\cdot}61$). Two responder and two non-responder patients had abnormally low percentages (and absolute numbers) of T cells of suppressor/cytotoxic phenotype, ranging from 16 to 20% of total lymphocytes.

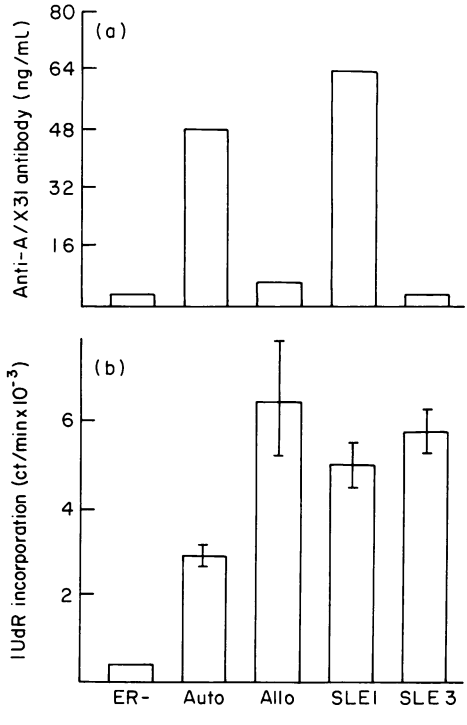

Fig. 3.(a) Specific anti-A/X31 antibody production and (b) MLR response, by autologous (Auto), allogeneic normal (Allo), and SLE (SLE1 and 3) cell mixtures.

Table 2. Total and A/X31 specific IgG production by normal non-T cells (normal A) cultured with non-irradiated and irradiated autologous (normal A), allogeneic normal (normal B) and allogeneic SLE (SLE 1 and 2) T cells.

T lymphocyte donor	ER$^-$ cell donor	Antigen	T cell irridiation	Response (ng/ml) Total Ig	Specific Ig
Normal A	Normal A	−	−	122	0
Normal A	Normal A	+	−	75	69
Normal B	Normal A	+	−	162	5
Normal B	Normal A	+	+	28	25
SLE 1	Normal A	+	−	156	72
SLE 1	Normal A	+	+	58	59
SLE 2	Normal A	+	−	445	71
SLE 2	Normal A	+	+	79	74

Total immunoglobulin production

Total IgG supernatants from allogeneic collaborations was measured by an ELISA assay. Results from one experiment are shown in Table 2. As previously reported, supernatants from alloactivated cultures contain significant amounts of polyclonal antibody. However, the specific anti-influenza antibody produced in virus stimulated cultures of non-irradiated SLE T cells with allogeneic normal non-T cells cannot simply reflect this polyclonal B cell activation. Table 2 also illustrates that although low dose T cell irradiation lowers total antibody production in these allogeneic combinations, it does not affect the amount of specific anti-influenza antibody produced.

Autoantibody production

Supernatants from the cultures were also screened for anti-DNA antibodies using an ELISA assay. No supernatants irrespective of origin, contained anti-DNA antibodies.

DISCUSSION

Two previous influenza immunization studies with SLE patients have both reported an absent antibody response to virus challenge *in vitro* by the SLE PBL (Mitchell *et al.*, 1982; Pelton & Denman, 1982). In normal non-responding donors, the lack of *in vitro* antibody response has been atrributed to a deficiency in the non-T cell compartment; irradiated T cells from normal non-responders help allogeneic normal responder non-T cells. This suggests that, following immunization or antigen exposure, responder non-T cells are removed from the circulation more rapidly than responder T cells. Rapid sequestration of antigen primed non-T cells would, however, be surprising in SLE, where there is good evidence for an increased proportion and absolute number of circulating B cells and plasma cells (Blaese, Grayson & Steinberg, 1980).

Since we were unable to study the B cell effector arm of the response in immune SLE patients, we have concentrated on helper T cell responses. In normal donors, T cells irradiated at low dose, or exclusively of helper/inducer phenotype, provide specific help for allogeneic normal non-T cells, and their frequency in the circulation is similar to that of conventional restricted T helper cells. Similarly, in SLE patients, irradiated T cells provide help for MLC mismatched normal responder non-T cells, irrespective of whether the patients had been intentionally immunized or not.

Low dose irradiated T cells from normal and lupus donors, therefore, behave similarly in providing specific allogeneic help. Unexpectedly, however, non-irradiated total T cells from 40% of the patients studied also helped MLC non-identical non-T cells, a phenomenon not observed with any normal or disease control donor. While others have shown, in mice, specific T cell proliferation to antigen presented on specific allogeneic haplotypes (Ishii 1981), we believe that this is the first report in man of specific antibody production by incompatible mixtures of total T and non-T cells.

The methods for demonstrating allogeneic T cell help in normal donors share the property of removing suppressor cells or preventing their generation. In SLE, suppressor T cell generation in response to mitogens, specific antigens, and in the autologous mixed lymphocyte reaction (MLC), is defective. In this study, the mean ratios of helper/suppressor T cells provided allogeneic help ('responders') were neither significantly different from the ratios in non-responder patients, nor from those in normal donors. Individual patients in both responder and non-responder groups, however, did have abnormal ratios, and some, reduced absolute numbers of suppressor/cytotoxic T cells. SLE T cells recovered from the allogeneic cultures were able to suppress PWM-induced polyclonal antibody and MLC responses, although neither they, nor T cells recovered from normal autologous cultures, specifically suppressed influenza antibody responses.

Clinical and pathological features of responder SLE patients were not obviously different from the non-responder group (Table 1). On average, responder patients were neither clinically more ill, receiving higher mean doses of steroids, nor had higher mean pathological indices of disease activity. Individual responder and non-responder patients had never taken steroids, and some were not receiving any therapy at the time of study. Moreover, four of the disease control donors were taking steroids, but remained, like all other disease control and normal donors, non-responders.

It has been suggested that autoantibody production is a consequence of abnormal T–non-T cell collaboration (Bretscher 1973). In mice, injection of LPS, a T cell dependent mitogen which does not require histocompatibility between T and non-T cells, leads to transient production of those autoantibodies characteristic of SLE (Fish & Ziff, 1982). Moreover, chronic graft versus host disease (GVHD), induced by injection of parental marrow into F_1 recipients, is also associated with chronic SLE like autoantibody production (Gleichmann, van Elvan & van der Veen, 1982). In man, chronic GVHD may also be associated with autoimmune disease, but with polymyositis rather than SLE (Pier & Dubowitz, 1983).

In the present study, we have demonstrated abnormal T–non-T cell interactions in SLE, but have failed to detect any consequent anti-DNA antibody production *in vitro*. Specific allogeneic helper T cells from some SLE patients have been cloned, and they, and the helper factors they produce, are being tested for their ability to induce anti-DNA and other autoantibodies from normal and SLE non-T cells.

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