Relationship of phytohaemagglutinin-induced lymphocyte transformation to disease activity in systemic lupus erythematosus

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Hughes, P., Holt, S., Rowell, N. R., and Dodd, J. K. (1976). *Annals of the Rheumatic Diseases*, **35**, 97–105. **Relationship of phytohaemagglutinin-induced lymphocyte transformation to disease activity in systemic lupus erythematosus.** Phytohaemagglutinin-induced lymphocyte transformation was studied in 19 patients with systemic lupus erythematosus (SLE) in relation to disease activity, peripheral blood lymphocyte count, serum iron and folate levels, and corticosteroid treatment. Similar studies were performed on a group of 28 age- and sex-matched controls and on 10 patients with facial palsy who were examined before and after 7 days of high-dose corticosteroid treatment.

The patients with SLE were found to have an impairment of lymphocyte transformation which was most marked in active stages of the disease and associated with a lymphopenia. This depressed transformation, which improved with the development of a remission, could not be attributed to the effects of corticosteroid treatment, inhibitory serum factors, iron deficiency, or any numerical reduction in blood lymphocytes, thus indicating along with evidence from other sources that SLE patients have a defect of cell-mediated immunity. The aetiological implications of these findings are discussed.

Systemic lupus erythematosus (SLE) is characterized by the presence of a wide range of autoantibodies (Holman, 1965). Little is known, however, of cellular immune function in this disorder despite the important co-operating and controlling effects that thymus-dependent (T) lymphocytes have on immune responses (Allison, Denman, and Barnes, 1971). Previous studies have produced evidence of cutaneous anergy both to tuberculin, streptokinase, candida, and mumps antigens (Toh and others, 1973) and to impaired mixed lymphocyte reactivity (Hedberg and others, 1971), suggesting that there may be a defect of cell-mediated immunity in SLE. The question of lymphocyte responsiveness to mitogens such as phytohaemagglutinin (PHA) has remained controversial with reports of both depressed (Bitter, Bitter, and Silberschmidt, 1971) and normal responses (Mellbye and others, 1973).

We have studied PHA-induced lymphocyte transformation in patients with SLE using the principle of the dose-response curve (Fitzgerald, 1971), con-

sidering also disease activity and other possible modifying factors such as iron deficiency, variation in lymphocyte count, and corticosteroid therapy. The findings indicate that patients with SLE have impaired lymphocyte transformation responses to PHA and an associated lymphopenia, both being most marked in patients with active disease and both perhaps being implicated in the pathogenesis of the disorder.

Materials and methods

PATIENTS

Nineteen patients with SLE (Medical Research Council, 1961), one of whom was male, mean age ($\pm$ SD) 38.2 ± 15.8 years, were studied. Disease activity at the time of testing was assessed on the basis of the following criteria.

Active disease

Patients who were ill and usually in hospital with varying combinations of fever, serositis, nephritis, haemolysis,

neurological manifestations, vasculitis, hypergamma-globulinaemia, anaemia, and an ESR >50 mm/h. This group consisted of 5 women, mean age ($\pm$ SD) 23.6 ± 5.9 years.

Slightly active disease

Patients troubled by intermittent minor symptoms such as myalgia or arthralgia, but without evidence of active visceral disease, anaemia, or grossly raised ESR. This group consisted of 7 women, mean age ($\pm$ SD) 37.5 ± 9.3 years.

Inactive disease

Patients now well and free of symptoms with no anaemia or raised ESR. This group consisted of 7 patients, one male, mean age ($\pm$ SD) 49.1 ± 16.1 years.

Two patients with active disease were not receiving treatment with corticosteroids at the time of the initial tests. Prednisone dosage in the remainder is shown in Table II.

CONTROLS

Two groups of controls were used. (1) 27 healthy volunteers (2 males), mean age ($\pm$ SD) 37.3 ± 11.4 years. (2) In order to assess the effect of corticosteroids on lymphocyte transformation, 10 patients with facial palsy (7 males), mean age ($\pm$ SD) 38.4 ± 10.3 years, were studied before and after 7 days' treatment with prednisone 60 mg/day.

Lymphocyte transformation was performed in both autologous plasma and human AB serum by a modification of the whole blood micromethod of Junge and others (1970). For studies in autologous plasma, one part of whole blood, anticoagulated with preservative-free heparin (Boots Pure Drug Co., Nottingham) 20 U/ml, was diluted with 9 parts TC199 (Wellcome Reagents Ltd., Beckenham), and aliquots (1 ml) of this mixture were then cultured in triplicate in stoppered siliconized tubes at 37°C for 72 hours with PHA (MR 10, Wellcome Reagents Ltd., Beckenham) at 0, 10, and 50 $\mu\text{g/ml}$. For studies in human AB serum, 2 ml heparinized blood were washed three times with 10 ml TC199 before finally being diluted 1 part in 9 with TC199 enriched with 5% human AB serum. Aliquots (1 ml) were then similarly cultured and stimulated with PHA.

(3H) Methyl-thymidine, 2 μCi (specific activity 5 Ci/mmol per l, Radiochemical Centre, Amersham), was added to each tube for the last 4 hours of culture and, after a preliminary lysis of red blood cells by 3% acetic acid, DNA was then precipitated by 5% trichloroacetic acid. The washed precipitate was solubilized with 1 mol/l hyamine in methanol (Nuclear Enterprises Ltd., Edinburgh), incorporated into NE 220 scintillator fluid (Nuclear Enterprises Ltd., Edinburgh), and counted in a Packard Tri-Carb liquid scintillation counter. Results were expressed as the $\log_{10}$ of the average count per minute (cpm) of triplicate cultures.

DESIGN OF INVESTIGATION

The lymphocyte response to PHA, together with some of the factors capable of modifying it, was investigated as follows. (1) Transformation response in SLE patients was always tested in both autologous plasma and human AB serum. (2) Transformation response in the controls with facial palsy was also tested in both autologous plasma and human AB serum before and after 7 days' corticosteroid

therapy. (3) Transformation response of normal controls was tested in autologous plasma only. (4) Inhibitory serum factors in SLE were also looked for by adding serum from 10 patients to replicate micromethod cultures from a single normal donor. The effect of the resulting 5% enrichment of the medium on the transformation response was observed. (5) The effect of variations in lymphocyte count on lymphocyte transformation was examined. The response in normal whole blood cultures, which had been diluted (1 in 19) to give lymphocyte counts comparable with those present in active SLE, was compared with that produced by the standard micromethod (diluted 1 in 9).

Absolute lymphocyte counts were calculated from total and differential leucocyte counts. Serum folate levels were estimated by the method of Millbank and others (1970). Serum iron levels were estimated by Autoanalyser. *Lymphocytotoxins*. Sera from 11 patients with SLE were examined for lymphocytotoxins (Ooi and others, 1974) using a panel of lymphocytes normally used for tissue typing.

STATISTICAL METHODS

As the variances of the lymphocyte transformation responses in some of the groups examined were not comparable by Student's 't' test, the data were analysed by the Wilcoxon two-sample ranking test (Sokal and Rohlf, 1969). Comparison of absolute lymphocyte counts, serum iron and folate levels was made using Student's 't' test.

Results

LYMPHOCYTE TRANSFORMATION

Lymphocyte transformation responses to 10 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ PHA in SLE patients and in the control groups are shown in Table I and Figs. 1–6. Normal controls responded in a uniform way (Table I) and the reproducibility of the method is illustrated by the serial observations made on one normal control (Fig. 5). Patients with SLE responded with more variation due to differences in the activity of their disease (Table I).

Active SLE

Lymphocyte transformation in these patients was markedly and significantly reduced at both concentrations of PHA in both human AB serum (PHA 10 $\mu\text{g/ml}$, $t = 3.47$, $P < 0.01$; PHA 50 $\mu\text{g/ml}$, $t = 2.51$, $P < 0.02$) and autologous plasma (PHA 10 $\mu\text{g/ml}$, $t = 3.47$, $P < 0.01$; PHA 50 $\mu\text{g/ml}$, $t = 3.50$, $P < 0.01$) when compared with the corresponding normal control values (Table I, Fig. 1). Although the response in human AB serum of this group appeared to be greater than in autologous plasma the difference was not significant ($P > 0.1$ at PHA 10 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$).

Serial observations made on one patient in this group are shown in Fig. 7. Lymphocyte transformation before corticosteroid treatment when the patient was ill with fever, haemolytic anaemia, pleurisy, and

Table I Lymphocyte transformation responses and blood lymphocyte counts in patients with SLE and in controls

Group studied	No.	Age (years) (mean $\pm$ SD)	Blood lymphocytes ($\times 10^9/l$) [†] (mean $\pm$ SD)	Lymphocyte transformation responses ($\log_{10}$ cpm) (mean $\pm$ SD)					
				Autologous plasma PHA concentration ($\mu g/ml$)			Human AB serum PHA concentration ($\mu g/ml$)		
				0	10	50	0	10	50
SLE*									
Active	5	23.6 $\pm$ 5.9	1.3 $\pm$ 0.33	2.5013 ± 0.2283	2.8908 ± 0.5496	3.6924 ± 0.3992	2.6131 ± 0.3217	3.4392 ± 0.4083	3.8594 ± 0.6410
Slightly active	7	37.5 $\pm$ 9.3	1.7 $\pm$ 0.77	2.3094 ± 0.2056	3.9151 ± 0.5432	4.3253 ± 0.4104	2.2831 ± 0.2264	3.9034 ± 0.2973	4.1878 ± 0.3253
Inactive	7	49.1 $\pm$ 16.1	2.4 $\pm$ 1.09	2.2121 ± 0.2109	4.1842 ± 0.1491	4.4475 ± 0.3146	1.9332 ± 0.2578	4.1082 ± 0.1756	4.5265 ± 0.0960
Normal controls	28	37.3 $\pm$ 11.4	2.3 $\pm$ 0.59	2.3262 ± 0.1737	4.5747 ± 0.2293	4.6897 ± 0.2139	—	—	—
Normal controls (with halved lymphocyte count) [†]	7	38.6 $\pm$ 13.3	1.3 $\pm$ 0.34	2.1789 ± 0.1626	4.4143 ± 0.3173	4.8336 ± 0.1827	—	—	—
One normal control									
Replicate cultures	5	45	2.5 $\pm$ 0.68	2.3536 ± 0.0879	4.6612 ± 0.1644	4.6653 ± 0.2983	—	—	—
Replicate cultures enriched with 5% SLE serum	10	45	2.5 $\pm$ 0.68	2.3067 ± 0.1746	4.1341 ± 0.4287	4.5546 ± 0.2322	—	—	—
Facial palsy									
Presteroid treatment	10	38.4 $\pm$ 10.3	2.0 $\pm$ 0.48	2.2488 ± 0.1402	4.3625 ± 0.2454	4.6738 ± 0.2839	2.2378 ± 0.1226	4.3681 ± 0.2009	4.6325 ± 0.1203
Poststeroid treatment	10	38.4 $\pm$ 10.3	2.9 $\pm$ 0.91	2.5014 ± 0.2884	4.2089 ± 0.2595	4.5737 ± 0.2846	2.5222 ± 0.2076	4.2441 ± 0.3844	4.5965 ± 0.2269

* Grades of activity as defined in text.

[†] See Methods.[‡] Conversion: SI to Traditional Units—Lymphocytes: $4.0 \times 10^9/l \approx 4000/mm^3$.

Raynaud's phenomenon, was grossly depressed but then rose towards normal as the disease went into a clinical and serological remission over 9 months with prednisone therapy.

Slightly active SLE

Although not as depressed as in active SLE, lymphocyte transformation in these patients was once more significantly impaired at both concentrations of PHA in both human AB serum (PHA 10 $\mu g/ml$, $t = 3.68$, $P < 0.001$; PHA 50 $\mu g/ml$, $t = 3.33$, $P < 0.001$) and autologous plasma (PHA 10 $\mu g/ml$, $t = 2.85$, $P < 0.01$; PHA 50 $\mu g/ml$, $t = 2.03$, $P < 0.02$) when compared with the response of normal controls (Table I, Fig. 2).

Inactive SLE

Lymphocyte transformation in these patients was much nearer to the normal range than in either of the

two groups above. Nevertheless, when compared with normal controls, the responses were still significantly impaired at 10 $\mu g/ml$ PHA in both autologous plasma ($t = 3.43$, $P < 0.01$) and human AB serum ($t = 3.09$, $P < 0.01$) and at 50 $\mu g/ml$ PHA in autologous plasma ($t = 2.32$, $P < 0.05$). The response at 50 $\mu g/ml$ PHA in human AB serum, however, was not significant ($t = 1.88$, $P > 0.05$) (Table I, Fig. 3).

Facial palsy group

Lymphocyte transformation in these patients, before steroid treatment, was slightly but significantly impaired at 10 $\mu g/ml$ PHA ($t = 2.31$, $P < 0.05$) but did not differ from the normal control response at 50 $\mu g/ml$ PHA ($t = 0.12$, $P > 0.1$). Treatment with prednisone (60 mg/d for 7 days) resulted in a reduction of the response at both concentrations of PHA in both human AB serum and autologous plasma but

FIG. 1 *Phytohaemagglutinin (PHA)-induced lymphocyte transformation in SLE. Dose-response curves (mean $\pm$ 2 SD) in autologous plasma (—) and human AB serum (.....) in 5 patients with active SLE compared with the response of 28 normal controls in autologous plasma (hatching)*

FIG. 2 *PHA-induced lymphocyte transformation in SLE. Dose-response curves (mean $\pm$ 2 SD) in autologous plasma (—) and human AB serum (.....) in 7 patients with slightly active SLE compared with the response of 28 normal controls in autologous plasma (hatching)*

this degree of impairment was not, surprisingly, significant ($P > 0.1$ at PHA 10 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$) (Table I, Fig. 4).

Inhibitory serum factors

Enrichment of a series of replicate lymphocyte cultures from one normal control with serum from 10 SLE patients (3 of whom had active disease) failed to produce a significant reduction in the transformation of the normal lymphocytes at 50 $\mu\text{g/ml}$ PHA, either when compared against the

replicate responses of the original donor ($P > 0.1$) or against the response of the whole normal control group ($t = 1.46$, $P > 0.1$). There was, however, a significant reduction in the transformation response of normal lymphocytes at 10 $\mu\text{g/ml}$ PHA when compared with the whole of the normal control group ($t = 3.22$, $P < 0.01$) (Table I, Fig. 5), but which was not significant when compared with the replicate responses of the original donor ($P > 0.1$). This isolated instance of an apparent impairment of the transformation response of normal lymphocytes by

FIG. 3 *PHA-induced lymphocyte transformation in SLE. Dose-response curves (mean $\pm$ 2 SD) in autologous plasma (—) and human AB serum (.....) in 7 patients with inactive SLE compared with the response of 28 normal controls in autologous plasma (hatching)*

FIG. 4 *PHA-induced lymphocyte transformation in 10 patients with facial palsy. Dose-response curves (mean $\pm$ 2 SD) in autologous plasma before (.....) and after (—) 7 days' treatment with prednisone 60 mg/day, compared with the response of 28 normal controls in autologous plasma (hatching)*

FIG. 5 Modification of PHA-induced lymphocyte transformation by SLE serum. Dose-response curves (mean $\pm$ 2 SD) in autologous plasma of 5 replicate cultures of one normal control (—) compared with (a) 10 similar cultures enriched with 5% serum from patients with SLE (.....), and (b) 28 normal controls (hatching)

serum from patients with SLE does not provide sufficient evidence that the impaired response of patients, particularly those with active disease, was due to the operation of inhibitory serum factors. Individual results, in fact, showed that any depression of transformation was usually only produced by sera from patients whose own responses were not markedly impaired, whereas the sera of patients with gross impairment of transformation had little or no effect on the response of normal lymphocytes. This situation is best exemplified by one patient (Fig. 7) whose lymphocyte transformation response was grossly impaired at a time when her serum had no effect whatsoever on the transformation response of normal lymphocytes in spite of the presence of lymphocytotoxins.

Variation in lymphocyte count

A reduction of lymphocytes to the numbers found in patients with active SLE had no significant effect ($t = 1.62$, $P > 0.1$) on the transformation response of normal lymphocytes (Table I, Fig. 6).

ABSOLUTE LYMPHOCYTE COUNTS

The results of the mean absolute lymphocyte counts for patients with SLE and the control groups are shown in Table II. The patients with active SLE had a significant lymphopenia when compared with normal controls ($t = 3.79$, $P < 0.001$) and although the lymphopenia was less marked in patients with only slight disease activity, the reduction was nevertheless significant ($t = 2.12$, $P < 0.02$). The mean lymphocyte count of patients with inactive SLE did not, however, differ significantly from that of the normal controls ($t = 0.21$, $P > 0.1$).

SERUM IRON AND FOLATE LEVELS

The mean serum iron and folate levels for patients with SLE and the control groups are shown in Table II. Serum iron levels were significantly lower in patients with SLE than in controls ($t = 4.39$, $P < 0.001$) but did not differ significantly when the various subgroups of SLE were compared with each other. There was no significant difference in serum folate levels in any of the groups studied.

FIG. 6 PHA-induced lymphocyte transformation in normal controls. Comparison of dose-response curves (mean $\pm$ 2 SD) in autologous plasma of 28 normal controls (hatching) with cultures from 7 normal controls (—) diluted to give lymphocyte counts comparable with those present in patients with active SLE

FIG. 7 PHA-induced lymphocyte transformation in SLE; serial studies (PHA 10 μ g/ml) in one patient (—) during transition from untreated active disease to a clinical and serological remission, compared with the corresponding responses (mean $\pm$ 2 SD) of 28 normal controls (hatching)

Table II Serum iron and folate levels and corticosteroid dosage in patients with SLE and controls

Group studied	Serum iron ($\mu\text{g/ml}$) (mean $\pm$ SD)	Serum folate ($\mu\text{g/ml}$) (mean $\pm$ SD)	Prednisone (mg/day) (mean $\pm$ SD)
SLE*			
Total group	79.0 $\pm$ 23.5	7.5 $\pm$ 4.9	9.5 $\pm$ 6.6
Active	77.6 $\pm$ 24.0	9.6 $\pm$ 8.1	20†
Slightly active	82.1 $\pm$ 27.3	6.0 $\pm$ 1.0	8.9 $\pm$ 5.0
Inactive	76.8 $\pm$ 22.6	7.1 $\pm$ 3.8	8.2 $\pm$ 3.7
Normal control	112.8 $\pm$ 48.9	8.5 $\pm$ 3.2	0
Facial palsy			
Presteroid treatment	107.7 $\pm$ 31.2	9.4 $\pm$ 3.1	0
Post-steroid treatment	—	—	60

* Grades of activity as defined in text.

† Two patients were untreated at the initial tests; the remaining 3 patients each received 20 mg prednisone/day.

LYMPHOCYTOTOXINS

Lymphocytotoxins, which were optimally active at 15°C, were detected in 4 of the 11 sera examined. A strong reaction was, however, found with only one serum (patient in Fig. 7) while the remaining 3 sera showed only weak lymphocytotoxic activity.

Discussion

This investigation has shown that patients with SLE have an impaired lymphocyte transformation response to PHA. This impairment was most obvious when suboptimal stimulatory doses of PHA were used and was marked in those patients with active disease. Although much less impairment was seen in patients whose disease was relatively quiescent, transformation responses were still significantly depressed even in patients in complete remission. These findings confirm and extend earlier published reports (Bitter and others, 1971; Rosenthal and Franklin, 1973; Paty and others, 1974) and together with the cutaneous anergy noted (Block and others, 1968; Toh and others, 1973), suggest that patients with SLE have an impairment of at least some aspects of cell-mediated immune function. The association of depressed transformation responses with both lymphopenia and disease activity is a particularly interesting aspect of this investigation as is the serial improvement in the PHA-responsiveness noted in one of the patients during a clinical and serological remission (Fig. 7).

Several factors could be involved in the depressed lymphocyte transformation observed. Firstly, the inhibitory effect of corticosteroids (Elves, Gough, and Israels, 1964; Heilman, Gambrill, and Lechner, 1973) should be considered as so many of the patients under investigation were receiving treatment with

these drugs. It is unlikely, however, that the major part of the impaired lymphocyte responsiveness seen in patients with active disease can be attributed to such therapy. Corticosteroid treatment of the control group with facial palsy never produced as profound a depression of lymphocyte transformation as that seen in active SLE. Moreover, patients with clinically quiescent SLE had higher lymphocyte transformation responses than those with more active disease despite receiving a similar mean dose of prednisone (Table II). Further evidence was provided by the 2 patients with active disease both of whom had depressed lymphocyte transformation before treatment, serial studies showing an increasing transformation response in one as the disease went into remission during corticosteroid therapy (Fig. 7). A similar impairment of lymphocyte transformation in untreated active disease (Bitter and others, 1971) with improvement in response during onset of a remission (Rosenthal and Franklin, 1973) was also noted in two earlier studies.

Secondly, the effect of inhibitory serum factors (Cooperband and others, 1968; Milton, 1971) should also be considered, particularly as lymphocytotoxins (Terasaki, Mottironi, and Barnett, 1970; Statsny and Ziff, 1971) with relative T-lymphocyte specificity (Lies, Messner, and Williams, 1973) have been found with a high frequency in the serum in SLE. The experiments which involved the addition of patients' serum to control cultures and the parallel use of human AB serum cultures all failed, however, to show the presence of any inhibitory serum factors which could account for the markedly depressed lymphocyte responses found in active disease. Moreover, lymphocytotoxins are optimally active at 15–22°C and have little or no activity at the temperatures present either in the whole blood cultures or

in vivo (Terasaki and others, 1970; Ooi and others, 1974). This point is illustrated by the serum of the patient in Fig. 7, which contained lymphocytotoxins but which failed to impair the transformation response of normal lymphocytes.

Another important factor is iron status as there is evidence that lymphocyte responsiveness is depressed by iron deficiency (Joynson and others, 1972). Although the mean serum iron level in all the patients with SLE was significantly lower than in any of the control groups, the patients with active disease and the most depressed lymphocyte transformation had mean serum iron levels that did not differ significantly from those found in patients in remission with much higher responses. We do not think, therefore, that iron deficiency can account for our findings.

The final possibility is that the impaired lymphocyte responsiveness to PHA present in SLE may be due to a quantitative and/or qualitative defect in the lymphocytes. A striking feature of this investigation has been that both disease activity and depressed lymphocyte transformation in SLE have been paralleled by a reduction in the peripheral blood lymphocyte count. Although a reduction of *normal* blood lymphocytes to the numbers present in active SLE did not produce any impairment of the lymphocyte transformation response, there is evidence that lymphocyte subpopulations in SLE are no longer normal. Messner, Lindström, and Williams (1973) reported a deficiency of T-lymphocytes in the disorder, and such a deficiency could, therefore, underlie the depressed transformation responses noted by us.

However, the gross impairment of transformation seen in some patients with active disease, amounting at times to almost complete unresponsiveness (Fig. 7), suggests that there is also likely to be some qualitative defect in the lymphocytes in these cases. Such immunodepression might be purely a secondary effect, analogous to the general depression of cell-mediated immunity that can result from the antigenic competition of graft versus host disease (Lapp and Möller, 1969; Möller, 1971). Alternatively, it may indicate some derangement of immunological mechanisms such as the loss of T-lymphocyte suppressor function that has been postulated to underlie autoimmune reactions (Allison, 1973). If either were the case then a similar reduction in lymphocyte responsiveness to mitogens might be expected in other situations involving autoimmunity. Evidence is still incomplete, but impaired lymphocyte transformation has been reported in Sjögren's syndrome, rheumatoid arthritis, and chronic active hepatitis (Leventhal, Waldorf and Talal, 1967; Toh and others, 1973), while a rising incidence of autoantibodies (Hill, 1961; Beck, 1963) and decreasing lymphocyte responsiveness to mitogens (Roberts-Thomson and others, 1974) are both associated with increasing age.

Finally, both depressed lymphocyte transformation (Salaman, 1970) and a reduction in circulating T-lymphocytes (Wybran and Fudenberg, 1973) have been recorded as transient features of certain virus infections, and the similarity of these observations to the findings of the present study are striking. Although doubt has been expressed about the true virus nature of the virus-like particles present in the lymphocytes and endothelial cells in SLE (Pincus and others, 1970), there is some evidence for a link with virus infection in the disorder. Patients with SLE have an increased incidence of high titre antibodies to several viruses (Evans, Rothfield, and Niederman, 1971; Hollinger and others, 1971; Hurd and others, 1972; Phillips and Christian, 1973; Laitinen and Vaheri, 1974) some of which are known to produce a temporary depression of cell-mediated immunity during infection (Starr and Berkovich, 1964; Rubin, 1966; Olson and others, 1968; Sheldon and others, 1973). In addition, the antibodies against RNA that are also frequently present in SLE are directed against the double-stranded form which is only found in large amounts in mammalian tissue if it is infected by an RNA virus (Schur and others, 1971). In experimental animals the lupus-like autoimmune disease of the NZB mouse is known to be associated with persisting infection with murine leukaemia virus (Levy and Pincus, 1970; Mellors and others, 1971) and as these animals age and develop autoimmune manifestations the T-lymphocytes show signs of deteriorating function, including impairment of the transformation response to PHA (Rodey, Good, and Yunis, 1971). These results and the recent report of an apparently selective impairment of cell-mediated immunity to measles virus in SLE (Utermohlen and others, 1974), coupled with increasing evidence to support a general cellular immune defect especially in active stages of SLE, should stimulate further investigation into the part played by virus infections in the pathogenesis of the disease.

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