A DEPRESSION OF CELL-MEDIATED IMMUNITY TO MEASLES ANTIGEN IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS*

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Considerable indirect data suggest that viruses may be of etiologic importance in systemic lupus erythematosus (SLE). Electron microscopic studies have demonstrated the presence of tubulo-reticular structures resembling paramyxoviruses in the cytoplasm of many different cell types from SLE patients, including lymphocytes (1–4), although their viral nature or derivation remains controversial. Other studies have attempted to define by serological means the association of a particular virus or a group of viruses with this disease (5–8). Antibody levels were found to be elevated to a number of viruses, but it has been difficult to separate specific antibody increase from the polyclonal elevation of immunoglobulins characteristic of these patients (7). Using a new approach to the question of viruses in SLE, we have examined cell-mediated immunity to several viruses using the direct leukocyte migration inhibition assay. Because most studies have shown antibody elevation to measles, rubella, and parainfluenza type 1, these were chosen as antigens. A specific depression of direct leukocyte migration inhibition was found to the measles antigen in patients with SLE as compared with controls, while normal inhibition to migration occurred with rubella and parainfluenza type 1 in these patients.

Materials and Methods

Patients.—17 unselected patients from the outpatient clinic of The Rockefeller University Hospital were studied. Except for one patient (Mal) with discoid lupus, all met the preliminary criteria of the American Rheumatism Association (9) for diagnosis of SLE. A summary of the pertinent clinical data is included in Table I. The controls were healthy unrelated normals.

Direct Leukocyte Migration.—The method of direct migration inhibition of peripheral blood leukocytes (DMI) as originally described by Bendixen (10) and modified as described earlier (11) was used in all experiments.

Antigens.—All antigens were obtained from Flow Laboratories, Inc., Rockville, Md. The

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measles virus (lot nos. M944034 and M944036, complement fixation (CF) titer 1:32) was the Edmonston 84F strain, final passage being in a primary African green monkey kidney culture with BME (Earle's salts) and 2% fetal bovine serum. The activated antigen preparation was prepared by freeze-thawing of virus infected cells, followed by sonication of the disrupted cells, and mild centrifugation to remove large cellular debris. The supernatant fluid containing measles antigens was used in the test system. Type 1 parainfluenza antigen (lot no. E921036 CF titer 1:32) was from the Sendai strain and was grown in embryonated hens' eggs (allantoic fluid). The Rubella antigen (CF titer 1:16) was prepared as an alkaline extract of rubella-(Gilchrist) infected BHK-21 (C/13) cell cultures and reconstituted in the medium described above (Flow Laboratories, Inc.). Lot nos. L960133 and C961207 were used. All antigen preparations, which contained no preservatives, were stored at −60°C. Appropriate dilutions were made immediately before use. Control antigens (i.e., supernates from the appropriate tissue culture lines but without virus, also provided by Flow Laboratories, Inc., were added to the medium in parallel with test antigens, to create the control fans.

Cytotoxicity Assay.—Antibodies to lymphocytes were determined in the serum of each patient by a two step microcytotoxicity assay as previously described (12). Maximum sensitivity was obtained by performing the assay at 15°C. Sera were tested against a panel of 25 normal and SLE peripheral blood lymphocytes, as well as with autologous lymphocytes in many cases. Strength of cytotoxic antibodies is expressed as strength index (the number of strongly positive assays in which more than 70% of lymphocytes were killed, divided by the total number of positive assays × 100).

RESULTS

The patients with SLE showed a marked lack of responsiveness to measles antigen as compared with the normal controls (see Fig. 1). The mean migration inhibition index to measles antigen at a 1:10 dilution was −19.3% (SE ± 4.9%) for the patients, against −47.6% (SE ± 3.3%) for controls ($P < 0.001$ by Student's t test). At 1:100 dilution of the measles antigen, the same difference held: −4.6% (SE ± 3.2) for the patients and −26.2% (SE ± 3.2%) for the controls ($P < 0.001$ by Student's t test). However, SLE and normal leukocytes did not differ in their response to either rubella or to parainfluenza type 1 antigens ($P > 0.6$ for rubella 1:40, and $P > 0.95$ for parainfluenza 1:10 and $P > 0.1$ at 1:100; see Fig. 1).

The degree of unresponsiveness to measles antigen did not show any obvious correlation with any specific clinical feature of the disease (e.g., nephritis) nor did it correlate with the use of immunosuppressive therapy (see Table I). In addition, the patients on immunosuppressive therapy showed a normal inhibitory response to the rubella and parainfluenza antigens. Only one patient (Hen) was unresponsive to all the antigens tested.

Antibodies to lymphocytes were commonly present (12/15 patients; see Table I) when sera were examined against a panel of normal peripheral blood lymphocytes. 6 of 12 sera exhibited autologous lymphocytotoxicity, although this could only be demonstrated by performing the assay at 15°C. Cytotoxic activity was, with one exception (Hen), not present at 37°C. It is interesting to speculate whether this patient's failure to respond to any of the antigens is secondary to the presence of this autocytotoxic antibody.

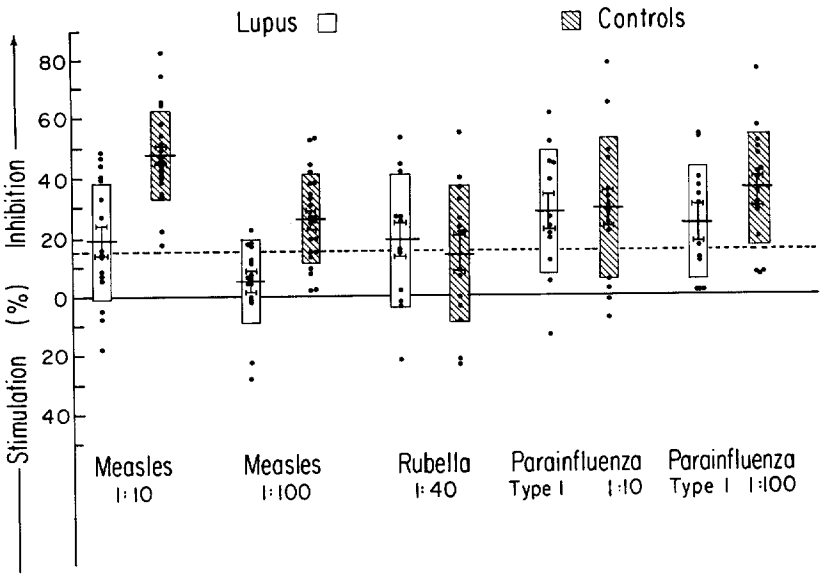

FIG. 1. A comparison of the migration inhibition values to viral antigens in patients with systemic lupus erythematosus and normal subjects.

Seven patients with rheumatoid arthritis and one patient with Sjögren's syndrome were studied using the measles and the parainfluenza type 1 antigens. The patient with Sjögren's syndrome showed normal responsiveness to the measles antigen, and a somewhat depressed response to the parainfluenza antigen at a 1:100 dilution. Four of the patients, with long-standing rheumatoid arthritis, showed no response to either antigen. One patient responded in a normal fashion to both antigens. Two patients responded with low inhibition (<30%) to both antigens.

DISCUSSION

In these experiments, a specific failure of leukocytes from SLE patients to respond to measles antigen was detected in the direct migration inhibition test, whereas a normal inhibitory response was seen with these patients' leukocytes to both parainfluenza type 1 and rubella antigens. We are currently extending these tests to include mumps antigen, and preliminary evidence indicates that the reaction of SLE patients to this antigen is normal as well.

Neither disease activity, as judged clinically and serologically, nor immunosuppressive therapy showed an obvious correlation with the degree of failure of migration inhibition to the measles antigen. Lymphocytotoxic antibodies do not seem to be responsible for this phenomenon, as there was no relationship between the presence of antibodies to lymphocytes as measured by the lymphocytotoxic assay and the failure of migration inhibition to measles antigen. This

TABLE I

Correlation of Migration Inhibition to Measles Antigen of SLE Leukocytes with Lymphocytotoxic Antibodies, Clinical Parameters, and Therapy

Patient	Measles migration inhibition index	Cytotoxicity strength index	Autologous lymphocytotoxicity (% cells killed)	$C'H_{50}$*	Anti-DNA (% C^{14} DNA binding) ¶	ESR‡	Clinical disease activity	Therapy	
								Prednisone	Azathioprine
								mg/d	*mg/d*
His	+17.2§	38	0	113	32	94	+	10	—
Mal	+7.9	0	ND	200	30	28	−	—	—
Esp	+4.2	70	0	127	41	30	+	20	—
Hen	−5.8	50	0	85	43	39	−	—	—
Wzx	−7.1	ND‖	ND	122	39	50	−	—	—
San	−8.7	8	60	99	22	42	−	15	—
Abb	−12.8	0	0	189	46	17	−	5	50
McI	−13.6	80	90	141	35	55	+	10	50
See	−14.9	0	ND	100	14	2	−	15	75
Nap	−15.8	ND	ND	175	23	ND	+	10	—
Won	−27.0	72	70	125	42	70	+	20	75
Ite	−33.4	100	90	182	27	67	+	20	—
Hars	−39.1	6	0	138	44	12	−	—	—
Tit	−40.3	26	50	211	50	111	−	—	—
Car	−44.0	17	ND	140	57	44	−	15	—
Harv	−46.3	34	0	114	55	92	+	30	—
DiP	−49.0	67	90	104	30	25	+	—	—

* CH5O, normal > 150.

‡ ESR, erythrocyte sedimentation rate.

§ +, % stimulation (control fans smaller than antigen fans).

‖ ND, not done.

¶ Upper limit of normal DNA binding, 26%.

lack of relationship also obtained when autologous cytotoxic reactions alone were considered.

Whether noncytotoxic antibodies specific for measles antigen, perhaps analogous to those shown to block the mixed lymphocyte reaction in SLE (14), are responsible for this failure of inhibition remains a possibility. It has been shown that lymphocytes from SLE patients may be coated with immunoglobulins even after extensive washing (R. Winchester et al., manuscript in preparation). However, preliminary experiments in which the assay was performed with leukocytes from normal donors in the presence of lupus serum (instead of fetal calf serum) have failed to reproduce the specific unresponsiveness to measles antigen seen with lupus cells.

The specificity of the failure of inhibition of migration to measles antigen would seem to argue against this being due to a generalized defect of cellular immunity in SLE for which there is certain evidence (15). However, the possibility remains that reactivity to measles antigen in this test system repre-

sents a more sensitive index of such a defect than reactivity to the other viral antigens.

Leukocyte migration values seen in patients with SLE closely parallel those seen in patients with multiple sclerosis (MS) (11), but are different from those obtained with leukocytes from patients with rheumatoid arthritis. The data thus far obtained suggest the possibility that failure of response to measles antigen may be peculiar to both MS and SLE. In spite of the obvious clinical differences between SLE and MS, there are points of basic similarity: (*a*) a viral etiology has been postulated for both; (*b*) the presence of similar virus-like or virus-related particles has been demonstrated, in the brain of patients with MS (16), and in various tissues, including lymphocytes, in patients with SLE; (*c*) a defect in cell-mediated immunity has been suggested in both (15, 17). Whether this unresponsiveness to measles antigen is related to a general defect in cellular immunity, to a specific T-cell defect (e.g., absent or blocked viral receptors), to the presence of latent measles or closely related virus, or to an abnormal antibody response to measles antigen, is unclear at this time.

SUMMARY

Using the direct migration inhibition test, response to measles antigen in patients with systemic lupus erythematosus (SLE) was found to be decreased when compared with that of normal subjects. No alteration was observed in similar experiments using parainfluenza type 1 and rubella antigens. The specific decrease in measles antigen effect showed no obvious correlation with activity of SLE or with the presence of lymphocytotoxic antibodies. Whether the specificity of the decrease in reactivity is due to some particular relationship between the measles virus or antigen and SLE, or to the possibility that measles reactivity is a more sensitive indicator of a generalized defect of cell-mediated immunity, remains unclear.

BIBLIOGRAPHY

1. Fresco, R. 1968. Tubular (myxovirus-like) structures in glomerular deposits from a case of lupus nephritis. *Fed. Proc.* **27**:246.
2. Kawano, K., L. Miller, and P. Kimmelstiel. 1969. Virus-like structures in lupus erythematosus. *N. Engl. J. Med.* **281**:1228.
3. Haas, Y. E., and E. J. Yunis. 1970. Tubular inclusions of systemic lupus erythematosus. Ultrastructural observations regarding their possible viral nature. *Exp. Mol. Pathol.* **12**:257.
4. Grimley, P. M., J. L. Decker, H. J. Michelitch, and M. M. Frantz. 1973. Abnormal structures in circulating lymphocytes from patients with systemic lupus erythematosus and related diseases. *Arthritis Rheum.* **16**:313.
5. Phillips, P. E., and C. L. Christian. 1970. Myxovirus antibody in human connective tissue disease. *Science* (*Wash. D. C.*). **168**:982.
6. Hollinger, F. B., J. T. Sharp, M. D. Lidskey, et al. 1971. Antibodies to viral antigens in systemic lupus erythematosus. *Arthritis Rheum.* **14**:1.

7. Phillips, P. E., and C. L. Christian. 1971. Virus antibody studies in connective tissue diseases. *Arthritis Rheum.* **14:**180.
8. Hurd, E. R., W. Dowdle, H. Casey, and M. Ziff. 1972. Virus antibody levels in systemic lupus erythematosus. *Arthritis Rheum.* **15:**267.
9. Cohen, A. S., W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Ropes, L. E. Shulman, and S. L. Wallace. 1971. Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21:**643.
10. Bendixen, G. 1967. Specific inhibition of the *in vitro* migration of leukocytes in ulcerative colitis and Crohn's disease. *Scand. J. Gastroenterol.* **2:**214.
11. Utermohlen, V., and J. B. Zabriskie. 1973. A suppression of cell-mediated immunity in patients with multiple sclerosis. *J. Exp. Med.* **138:**1591.
12. Wernet, P., T. Feizi, and H. G. Kunkel. 1972. Idiotypic determinants of immunoglobulin M detected on the surface of human lymphocytes by cytotoxicity assays. *J. Exp. Med.* **136:**650.
13. Lawrence, H. S., and S. Al-Askari. 1971. The preparation and purification of transfer factor. *In In Vitro* Methods of Cell-Mediated Immunity. B. R. Bloom and P. R. Glade, editors. Academic Press, Inc., New York. 531.
14. Wernet, P., and H. G. Kunkel. 1973. Antibodies to a specific surface antigen of T cells in human sera inhibiting mixed leukocyte culture reactions. *J. Exp. Med.* **138:**1021.
15. Rosenthal, C. J., and E. C. Franklin. 1973. Deficiency of cell-mediated immunity (CMI) in active SLE: changing patterns after steroid therapy. *Arthritis Rheum.* **16:**565.
16. Watanabe, I., and H. Okazaki. 1973. Virus-like structures in multiple sclerosis. *Lancet.* **2:**569.
17. Hedberg, H., B. Källén, B. Low, and O. Nilsson. 1971. Impaired mixed leucocyte reaction in some different diseases, notably multiple sclerosis and various arthritides. *Clin. Exp. Immunol.* **9:**201.