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**Research Article**

The immunological responsiveness of a panel of 17 patients with systemic lupus erythematosus (SLE) was studied in an in vitro model of xenogeneic sensitization against mouse lymphoid cells. Generation of cytotoxic thymus-derived (T) cells evaluated by a chromium release assay against labeled target cells was found to be drastically impaired in these lupus patients. Such depression was independent of drug therapy at the time of the study, clinical status, and other immunological parameters such as antibodies against native DNA, complement levels, cryoglobulinemia, circulating immune complexes, or T- and bone marrow-derived (B)-cell numbers. In contrast to the cytotoxic response, the proliferative responses to phytohemagglutinin, to allogeneic lymphocytes, and to xenogeneic lymphocytes were not significantly different from those of normal individuals. The latter response was shown to be H-2 restricted with the primed lymphocyte test. These results suggest the presence of a selective defect in the generation or in the expression of killer cells rather than a deficiency in antigen recognition by T cells. The role of serum factor(s) was examined by educating the lymphocytes of normal subjects in the presence of serum from SLE patients. Such manipulation affected both the generation of killer cells and the proliferative response. Finally our observations indicate that depression of cell-mediated immunity in SLE patients may be associated with several mechanisms including a cellular one, specifically […]



# Selective Depression of the Xenogeneic Cell-Mediated Lympholysis in Systemic Lupus Erythematosus

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ABSTRACT The immunological responsiveness of a panel of 17 patients with systemic lupus erythematosus (SLE) was studied in an in vitro model of xenogeneic sensitization against mouse lymphoid cells. Generation of cytotoxic thymus-derived (T) cells evaluated by a chromium release assay against labeled target cells was found to be drastically impaired in these lupus patients. Such depression was independent of drug therapy at the time of the study, clinical status, and other immunological parameters such as antibodies against native DNA, complement levels, cryoglobulinemia, circulating immune complexes, or T- and bone marrow-derived (B)-cell numbers. In contrast to the cytotoxic response, the proliferative responses to phytohemagglutinin, to allogeneic lymphocytes, and to xenogeneic lymphocytes were not significantly different from those of normal individuals. The latter response was shown to be H-2 restricted with the primed lymphocyte test. These results suggest the presence of a selective defect in the generation or in the expression of killer cells rather than a deficiency in antigen recognition by T cells. The role of serum factor(s) was examined by educating the lymphocytes of normal subjects in the presence of serum from SLE patients. Such manipulation affected both the generation of killer cells and the proliferative response. Finally our observations indicate that depression of cell-mediated immunity in SLE patients may be associated with several mechanisms including a cellular one, specifically affecting the generation of killer T cells, and a humoral one possibly as a result of antilymphocytic antibodies and(or) immune complexes.

## INTRODUCTION

The immunological etiology of systemic lupus erythematosus (SLE)[1] is now well-accepted (1–3), yet the precise nature of the immunological imbalance that leads to the disease onset is still hypothetical (4). Various types of autoantibodies binding to cellular components can be detected (5). It is likely that such antibodies play a significant role in the onset of some of the organ manifestations of the disease, such as nephritis or arthritis (6, 7). However, it is still not clear whether they represent the primary event in the pathogenesis of the disease, or rather a consequence of a more primitive defect that triggers off aberrant clones of autoantibody-producing cells (8, 9). The latter hypothesis has prompted numerous investigators to evaluate the thymus-derived (T)-cell-mediated responsiveness of SLE patients.

We have recently shown that human peripheral blood lymphocytes (PBL) can be educated against mouse cells to differentiate into H-2-specific cytotoxic T cells (10). This model offers the possibility to evaluate a well-defined T-cell function, namely the generation of killer cells, under conditions where the HLA haplotype of the responder should supposedly not interfere with the magnitude of the response. We have applied this model to the study of cellular immunological reactivity of 17 SLE patients, compared to 20 control subjects. We have observed a severe depression of xenogeneic cell-mediated lympholysis (CML) in all SLE patients contrasting with their ability to be normally stimulated by phytohemagglutinin, alloantigens or

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[1] *Abbreviations used in this paper*: CML, cell-mediated lympholysis; HBSS, Hanks' balanced salt solution; MLC, mixed-lymphocyte culture; PBL, peripheral blood lymphocytes; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes; [$^3$H]TdR, tritiated thymidine.

xenoantigens, and to respond specifically to the latter in a secondary mixed-lymphocyte culture (MLC) test.

## METHODS

### *Patients and control subjects (Table I)*

17 patients with SLE, defined by the American Rheumatism Association criteria (11) were compared with 20 control subjects: 13 normal individuals from the local bloodbank and 7 hospitalized patients (5 with chronic renal failure without hemodialysis and without treatment, and 2 untreated urological patients). All SLE patients were female: 4 presented a major exacerbation at the time of the test, 10 had a mild or moderate activity, and 3 could be considered as inactive. The degree of disease activity graded from one to four integrates both the number of organs involved and the severity of the lesions, and was independently evaluated by two physicians. All patients had shown major humoral abnormalities characteristic of SLE (anti-native DNA antibodies, low C3) in the past. Treatment at the time of the test consisted of prednisone and cyclophosphamide (three cases) or prednisone alone (five cases). Two patients received indomethacin and another one hydroxychloroquine sulfate. Six patients were not receiving medications at the time of the study. Among these six patients four (Patients 3, 7, 8, 11) had never received any treatment, whereas the other two had been off therapy for more than 6 mo when the investigation was performed.

### *Mononuclear cell isolation*

Peripheral blood was drawn aseptically into a 50-ml syringe (Terumo, Tokyo, Japan) containing preservative-free heparin (Liquemine, Roche, Paris, France), 10 IU/ml of blood. The blood was diluted at 1:3 with Hanks' balanced salt solution (HBSS) (Institut Pasteur, Paris, France). 30-ml aliquots were layered on 10 ml of a Ficoll-Angiocontrix mixture (1,077 specific density): 24 parts of 9% wt/vol Ficoll (Pharmacia, Uppsala, Sweden) and 10 parts of a 30% solution of iothalamate sodium (Angiocontrix, Guerbet, Paris, France) in 50-ml plastic conical tubes (2070, Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, California). The tubes were centrifuged at room temperature at 400 *g* for 20 min and the opalescent lymphocyte-enriched ring was pipetted off. Mononuclear cells were spun down at 600 *g* for 10 min, and the cell pellet washed three times in HBSS at 400 *g* for 5 min. The mononuclear cells were finally resuspended in culture medium and counted in Turk's solution. Cell viability assessed in the presence of a 0.2% solution of trypan blue (Grand Island Biological Co., Grand Island, N. Y.) was always over 95%. Percentage of mononuclear cells was not significantly different in SLE patients and in controls.

### *Preparation of sensitizing mouse spleen cells*

DBA/2 and C57BL/6 mice (male and female) were purchased from the breeding center of the Centre National de la Recherche Scientifique (Orléans la Source, France) and kept in our own facilities until use. No viral contamination or abnormal animal death was encountered over the study. Animals were fed with composite feeding (R.03 Usine d'Alimentation Rationnelle, Epinay, France) and water ad libitum. Spleens were aseptically removed and teased with fine forceps in Petri dishes (3002, Falcon Labware) containing HBSS. Clumps were allowed to sediment at room temperature and cell suspensions were passed through needles of different gauges (Terumo 1, 0.9 × 38 mm; 14, 0.65 × 32 mm; 25, 0.5 × 25 mm). The cells were then spun down at 400 *g* for 5 min and the majority of erythrocytes was removed by hypotonic shock (10 s in sterile distilled water). Cells were subsequently washed three times in HBSS, resuspended in HBSS, and incubated with mitomycin C (Ametycine, Choay, Paris, France) for 45 min at 37°C (50 μg/ml per $1 \times 10^7$ cells). After three consecutive washes in HBSS, an aliquot of the cell suspension was counted in Turk's solution. Viability assessed by trypan blue exclusion was always over 80%.

### *Tumor cell lines*

P 815 mastocytoma line (H-$2^d$) was maintained in vivo in ascitic form. DBA/2 mice were injected intraperitoneally with $1 \times 10^5$ cells in normal saline solution and used 10 d later. EL4 lymphoma line (H-$2^b$) was similarly maintained in C57BL/6 mice. Cells were pipetted from the mice peritoneum, and resuspended in saline, containing heparin (10 IU/ml). Tumor cells were washed twice in saline and used, after chromium labeling, as target cells in cytolysis assays.

### *In vitro sensitization of human PBL against mouse spleen cells*

Culture medium was RPMI 1640 supplemented with 2 mM l. glutamine, (Grand Island Biological Co.) penicillin (50 IU/ml) streptomycin (50 μg/ml), 10 mM, Hepes buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid from Grand Island Biological Co.), and 10% heat-inactivated (56°C for 45 min) pooled human AB serum. Human responder cells ($2 \times 10^7$ cells) were mixed with $2 \times 10^7$ mouse-stimulating spleen cells in a final vol of 20 ml in tissue culture flasks (3013, Falcon Labware). The flasks were left upstanding for 7 d at 37°C in a humidified atmosphere of 5% $CO_2$ in air. Cells were harvested by vigorous pipetting, washed once in HBSS, and resuspended in fresh culture medium. Cell count at the end of the culture was always over 60% of the initial value. Viability assessed by trypan blue exclusion was over 75%.

### *Xenogeneic MLC test*

Human PBL were diluted in culture medium (see above) at the concentration of $1 \times 10^6$ cells/ml; mitomycin C-treated mouse spleen cells were adjusted in the same medium to a final concentration of $1 \times 10^7$ cells/ml; 0.1-ml aliquots from each cell suspension were mixed in the wells of flat-bottomed tissue culture microplates (3040, Falcon Labware). The cell mixtures were cultured at 37°C in a 5% $CO_2$ humid atmosphere and pulsed at day 3 with 1 μCi of titrated thymidine ([$^3$H]TdR) (1 Ci/mM sp act) (Commissariat a l'Energie Atomique, Saclay, France). The well contents were harvested 16 h later with a Mash II apparatus (Microbiological Associates, Walkersville, Md.). The dried pieces of filter paper were put into scintillation liquid (Instagel, Packard Instrument Co., Zurich, Switzerland) and counted in a scintillation spectrometer (Searle Diagnostics Inc., Subsid. of G. D. Searle & Co., Des Plaines, Ill.). Cultures were all done in triplicate.

### *Restimulation test with primed lymphocytes*

Human PBL ($2 \times 10^7$ mononuclear cells) were cultured with $2 \times 10^7$ mitomycin c-treated mouse spleen cells, as described above for sensitization. 25% of the medium was replaced at days 5 and 7. At day 9, surviving cells were harvested from the flasks, washed once and resuspended in fresh culture medium. The viable cells were counted, dispensed

into microplate wells and restimulated for 2 d with mitomycin C-treated mouse spleen cells, according to the procedure described for the MLC test. Cultures were pulsed with 1 μCi of [$^3$H]TdR and harvested 6 h later.

### *CML assay*

Ascites fluid-containing tumor cells was drawn from the peritoneal cavity and immediately diluted into saline plus heparin (10 IU/ml). The tumor cell suspension was washed twice, and counted in Turk's solution. 5 million cells were labeled for 1 h at 37°C with 100 μCi of $^{51}$Cr (sodium chromate, Commissariat a l'Energie Atomique, sp act, 143 mCi/mg) in a final vol of 0.25 ml. Target cells were subsequently washed three times in HBSS and resuspended in culture medium; $1 \times 10^4$ chromium-labeled target cells were dispensed into each well of round-bottom microculture plates (Cooke Engineering, Alexandria, Va.) and various amounts of educated or noneducated PBL (from 100 effectors to 1 target down to 12.5/1) were added. Noneducated PBL were cultured alone for 7 d in the absence of mouse cells. The plates were incubated at 37°C in a 5% $CO_2$ humid atmosphere for 16 h. At the end of incubation, they were centrifuged at 600 *g* for 5 min at 4°C, and 100 μl of supernate was removed from each well and counted in a Packard Autogamma scintillation spectrometer. Maximum chromium release was measured after addition of detergent (RBS, Microbiological Associates). Spontaneous release, i.e., release from target cells incubated alone never exceeded 35% of the maximum chromium release. Percentage of specific release was calculated as follows: sensitized lymphocytes (counts per minute) − noneducated lymphocytes (counts per minute)/maximum chromium release (counts per minute) − spontaneous release (counts per minute) × 100. A regression curve was derived from the experimental values obtained at various effector target cell ratios and the results are given as the percentage of specific release corresponding to an effector target cell ratio of 50:1.

### *Routine tests for evaluation of immunological parameters in SLE patients*

*Transformation with phytohemagglutinin.* Control and patient PBL ($1 \times 10^5$ cells in 0.2 ml vol) were cultured in microculture plates for 3 d with various dilutions of phytohemagglutinin-M (Difco Laboratories, Detroit, Mich.). Culture medium consisted of complete RPMI 1640 supplemented with 10% heat-inactivated pooled human AB serum. Cells were pulsed at day 2 with 1 μCi of [$^3$H]TdR and harvested 16 h later, as described above.

*Allogeneic MLC test.* Human responding cells ($1 \times 10^5$) were cocultured with $2 \times 10^5$ mitomycin C-treated allogeneic cells. Responding cells were obtained from SLE patients or normal controls and stimulating cells from non-related human donors from the local blood bank. Stimulating cells were treated with mitomycin C, as described above for mouse spleen cells. Cell mixtures (total vol, 0.2 ml) were distributed into wells of microplates and left for 6 d at 37°C. Culture medium consisted of complete RPMI 1640 supplemented with 10% heat-inactivated pooled human AB serum. Cultures were pulsed at day 5 with 1 μCi of [$^3$H]TdR and harvested 16 h later.

*Erythrocyte (E) rosette formation (12).* E rosettes were formed by mixing $3 \times 10^7$ fresh sheep erythrocytes (SRBC) (Institut Pasteur, Paris, France) with $1 \times 10^6$ human PBL in HBSS supplemented with 20% human AB serum, previously absorbed volume to volume with packed SRBC. Cell mixtures were incubated for 30 min at 37°C, centrifuged for 5 min at 200 *g*, and stored overnight at 4°C before being gently resuspended on a roller (Apelab, Paris, France) and counted in a hematocytometer. Leukocytes surrounded by three or more SRBC were counted as rosettes.

*Erythrocyte-antibody-complement rosette formation (13).* SRBC were washed three times in HBSS, resuspended to 2% in HBSS and incubated volume to volume with diluted rabbit anti-SRBC serum (Institut Pasteur) at 37°C for 30 min. The antiserum dilution was determined according to its subagglutinating titer. The antibody coated SRBC were washed three times in veronal buffer at 800 *g* and mixed volume to volume with fresh mouse serum of CFI strain diluted 1:20 in HBSS. After 30-min incubation at 37°C and three washes in veronal buffer, the sensitized SRBC ($3 \times 10^7$) were mixed with $1 \times 10^6$ human PBL in HBSS. Tubes were incubated for 30 min at 37°C and rosette counting was done in a hematocytometer, after gentle resuspension on a roller.

*Anti-DNA antibody determination.* Antibodies to DNA were detected by a Farr test (14) using $^{14}$C-labeled DNA from *Escherichia coli.* (Amersham Corp., Arlington Heights, Ill.). Bindings above 20% were considered as abnormal.

*Determination of C3 and C4 serum levels.* Complement fractions C3 and C4 were measured using radial immunodiffusion plates (C3 immunoplates purchased from Hyland Laboratories, Los Angeles, Calif., and C4 immunoplates purchased from Behring Institute, Paris, France).

*Cryoglobulin determination.* The blood was drawn at 37°C and kept at this temperature until the serum was separated. The cryoglobulins were defined by the presence of a precipitate after 8-d storage at +4°C.

*Determination of circulating immune complexes (15).* Circulating immune complexes were determined by the technique of precipitation by polyethylene-glycol (PEG 6,000, Fluka, Buchs, Switzerland). 4 ml of a 7% polyethylene-glycol solution in borate buffer (0.1 M, pH 8.4) were mixed with 4 ml of serum diluted 1:25 in the same buffer. After 18 h incubation at 4°C, the tubes were centrifuged at 20,000 *g* for 20 min. The supernates were discarded and the precipitates dissolved in 5 ml of NaOH 0.1 N. The quantity of precipitated proteins was read by optical density at 280 nm with a spectrophotometer (Beckman Instruments, Inc., Paris, France). When abnormal precipitate was observed the amount of globulin and C4 present in the precipitate was evaluated as described (15).

## RESULTS

*CML test.* PBL from 16 out of the 17 SLE patients of our panel were tested for their ability to differentiate into cytotoxic T cells after xenogeneic sensitization in vitro. Results are shown in Fig. 1. Educated lymphocytes from the SLE patients showed an almost complete absence of cytotoxic reactivity. Such lack of responsiveness occurred with all SLE patients' lymphocytes, irrespective of their clinical, therapeutical, and immunological status (for details see Table I). We also tested PBL from 13 normal individuals belonging to the local blood bank and PBL from 7 non-SLE patients (5 with chronic renal failure without being hemodialyzed, and 2 with urological disorders but normal renal function). Among the 13 normal donors, 10 gave a significant cytotoxic response (Fig. 1). On three occasions, these PBL, educated against DBA/2 spleen cells (H-2$^d$), were also assayed against EL4 targets (H-2$^b$). No cytolysis was observed against the latter target (data not shown)

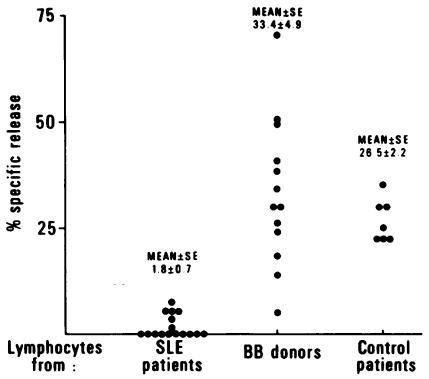


FIGURE 1 Cytotoxic response of xenoeducated human PBL. The percentage of specific release for each individual donor is given for an effector:target cell ratio of 50:1. Effector cells are PBL cocultured for 7 d with mitomycin C-treated DBA/2 (H-$2^d$) mouse spleen cells. Target cells are P 815 (H-$2^d$) mastocytoma cells. BB, local blood bank.

confirming the H-2 restriction already described in cell-mediated xenogeneic reactions (16–18). Educated PBL from three normal subjects showed only slight cytotoxicity against the relevant (H-$2^d$) target cells in spite of a normal viability of the recovered effector cells. In fact, we have already observed and reported that about 20% of normal donors are low responders in the xenogeneic assay (10). As for the seven non-SLE patients they all gave a consistent cytotoxic response not significantly different ($P > 0.05$ by Student's $t$ test) from that of the normal individuals (Fig. 1).

*MLC test.* The lack of efficient generation of cytotoxic T cells in SLE patient lymphocytes prompted us to determine whether this defect was associated with an absence of response in xenogeneic MLC. Lymphocytes from 6 out of the 16 SLE patients and from 6 normal controls were tested for thymidine uptake after 72 h of human-mouse mixed culture. As can be seen in Table II, the blastogenic response of SLE and normal PBL were not significantly different, suggesting that the CML defect in SLE patients was not related to an absence of lymphocyte proliferation after antigenic exposure. The antigen specificity of the blastogenic response was also checked for both SLE and normal PBL according to the method described by Lindhal and Bach (18). Namely anti-DBA/2-educated PBL were left for 9 d in culture, and then restimulated with H-2-compatible or -incompatible irradiated spleen cells. As

TABLE I
*Clinical and Immunological Findings in 16 Patients with SLE*

| Patients | Age | Sex | Disease activity* | SLE criteria positive‡ | Drug therapy: Prednisone | Drug therapy: Cyclophosphamide | DNA %B§ | C3‖ | C4¶ | IC** | Cryo.‡‡ | E. rosettes§§ | EAC rosettes§§ | PHA reactivity | Allogeneic MLC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *yr* | | | | *mg/d* | | % | | | | | % | % | | |
| 1 | 48 | F | 4 | 1,3,7,8,10,12,14 | 40 | 75 | 82 | 10 | 5 | + | + | 74 | 10 | $N^{al}$ | $N^{al}$ |
| 2 | 21 | F | 3 | 1,3,5,8,14 | 0 | 0 | 56 | 100 | 54 | – | + | 74 | 17 | $N^{al}$ | $N^{al}$ |
| 3 | 11 | F | 1 | 1,3,5,7 | 0 | 0 | 60 | 70 | 17 | + | + | 67 | 13 | $N^{al}$ | ND |
| 4 | 35 | F | 3 | 3,7,12,14 | 5 | 20 | 20 | 30 | — | – | – | 71 | 7 | | |
| 5 | 30 | F | 3 | 1,8,10,11 | 30 | 0 | 41 | 70 | 35 | – | – | 61 | 4 | $N^{al}$ | $N^{al}$ |
| 6 | 30 | F | 1 | 1,2,3,5,12,14 | 0 | 0 | 75 | 55 | 20 | + | + | 43 | 4 | ND | ND |
| 7 | 30 | F | 1 | 1,2,3,5,8,12 | 0 | 0 | 76 | 100 | 24 | – | – | 73 | 3 | $N^{al}$ | $N^{al}$ |
| 8 | 36 | F | 1 | 1,5,12,14 | 0 | 0 | 12 | 150 | 87 | – | – | 77 | 1 | $N^{al}$ | $N^{al}$ |
| 9 | 27 | F | 3 | 7,8,10,11,14 | Hydroxychloroquine sulfate 400 | | 75 | 100 | 30 | – | – | 86 | 3 | $N^{al}$ | $N^{al}$ |
| 10 | 35 | F | 3 | 1,4,7,8,10,11,12,14 | 5 | 20 | 73 | 115 | 20 | – | – | 72 | 4 | ND | ND |
| 11 | 54 | F | 2 | 8,10,11,14 | 0 | 0 | 14 | 115 | 42 | – | – | 72 | 2 | $N^{al}$ | ND |
| 12 | 29 | F | 2 | 1,2,4,5,8,12 | Indomethacin 75 | | 11 | 150 | 48 | – | – | 45 | 25 | ND | ND |
| 13 | 20 | F | 4 | 1,8,10,11,12,17 | 15 | 0 | 17 | 90 | 45 | – | – | 72 | 13 | ND | ND |
| 14 | 56 | F | 2 | 3,7,10,11 | Indomethacin 200 | | 12 | 160 | 37 | + | – | 75 | 3 | ND | ND |
| 15 | 20 | F | 4 | 1,8,10,11,12,17 | 15 | 0 | 17 | 95 | 50 | – | – | 70 | 10 | ND | ND |
| 16 | 30 | F | 3 | 4,7,8,10,11,12,14 | 20 | 0 | 27 | 120 | 70 | – | – | 84 | 4 | ND | ND |
| 17 | 21 | F | 3 | 3,5,7,8,10,12 | 60 | 0 | 80 | 30 | 15 | + | – | 65 | 5 | $N^{al}$ | $N^{al}$ |

ND, not done; PHA, phytohemagglutinin.
* Disease activity is over-all clinical evaluation of activity coded; 1, remission or no activity; 2, mild; 3, moderate; 4, severe activity.
‡ Criteria positive are preliminary American Rheumatism Association criteria positive by number as listed in reference 11.
§ DNA %B is percent serum binding of native DNA.
‖ C3 is third fraction of complement (normal range 80–120 mg/100 ml of serum).
¶ C4 is fourth fraction of complement (normal range 30–70 mg/100 ml of serum).
** Presence (+) or absence (–) or immune complexes (IC) by polyethylene-glycol precipitation.
‡‡ Presence (+) or absence (–) of cryoglobulins (Cryo).
§§ E and erythrocyte-antibody-complement rosettes marking, respectively, for T and B lymphocytes.
¶ $N^{al}$, normal response, meaning that no significant difference was encountered in counts per minute incorporation between SLE and control lymphocytes tested on the same day.

TABLE II
*Xenogeneic Mixed-Lymphocyte Culture Harvested at Day 4*

| Experiment | Responder cells* | Mouse-stimulating cells* | [3H]TdR uptake‡ |
|---|---|---|---|
| | | | *cpm±SE* |
| 1 | Blood bank donor | none | 755±12 |
| | | DBA/2 | 15,257±2,120 |
| | SLE No. 11 | none | 698±383 |
| | | DBA/2 | 9,842±799 |
| 2 | | | |
| | Blood bank donor | none | 499±19 |
| | | DBA/2 | 2,548±282 |
| | Urological patient | none | 766±80 |
| | | DBA/2 | 1,436±142 |
| | SLE No. 13 | none | 285±102 |
| | | DBA/2 | 2,979±405 |
| 3 | Blood bank donor | none | 321±17 |
| | | DBA/2 | 2,619±161 |
| | SLE No. 10 | none | 467±59 |
| | | DBA/2 | 1,361±77 |
| | SLE No. 16 | none | 636±219 |
| | | DBA/2 | 2,091±858 |

* $1 \times 10^5$ human PBL cocultured with $1 \times 10^6$ mouse spleen cells treated with mitomycin C.
‡ Thymidine incorporation measured at day 4.

shown in Table III, H-$2^d$ primed PBL responded strongly in 48 h to a restimulation with H-$2^d$ cells, and only weakly to a restimulation with H-$2^b$ lymphocytes. Both types of responder PBL from SLE or from normal donors showed the same restriction of specificity and the same intensity in their response.

*Effect of SLE serum upon xenosensitization.* It has been suggested that the T-cell abnormalities encountered in SLE patients might be consecutive to the effect of factors present in their serum, such as antigen-antibody complexes or autoantibodies directed against T lymphocytes (19–24). We first tested the effect of SLE serum on the proliferative response of PBL to lectins or allo- and xenoantigens. Lymphocytes from normal or SLE donors were cultured in medium supplemented with graded amounts of SLE serum. The final concentration of serum was maintained constant at 20% by complementation with normal AB serum. As shown in Table IV, 5% and 10% SLE serum caused a dramatic inhibition of proliferation of both normal and SLE lymphocytes. 1% serum was apparently insufficient to significantly modify the reaction. It should be noted that similar inhibition was also observed when SLE serum was tested on autologous SLE lymphocytes indicating that the effect could not be attributed to natural alloantibodies. The next step consisted in evaluating the effect of SLE serum on the generation of cytotoxic T cells from PBL of normal donors. The presence of 10% SLE serum during education resulted in a total abrogation of the cytotoxic response (Table V). On the other hand, preincubating the PBL with SLE serum before education in normal medium did not alter the response (Table V, experiment 3).

A total of five different SLE sera were tested in mixed lymphocyte cultures. All of them induced a severe inhibition of the reaction. Three of them tested in macroculture-generating xenogeneic cytotoxic cells also prevented the appearance of effector cells. Because the sera were obtained from patients either on or off therapy, and because some did contain, whereas others did not contain, abnormal levels of immune complexes and cryoglobulins, it is apparent that these factors are not directly involved in the inhibitory properties of SLE sera.

## DISCUSSION

This study demonstrates the occurrence of a striking impairment among SLE patients of their capacity to generate cytotoxic cells in vitro. Indeed, all patients examined disclosed an almost total unresponsiveness against xenogeneic target cells compared to the 80% good responders found among normal donors and non-SLE patients. This immunological abnormality seems, however, relatively restricted to this particular function because we found other immunological parameters such as T-cell number or T-cell stimulation by lectins to be within the normal range, an observation which is in agreement with several other reports. Some authors have reported minor T-cell abnormalities in SLE patients yet these were essentially described during exacerbation phases (25–27) and in this study only three patients were in such an active stage.

As a matter of fact, the literature dealing with the cell-mediated reactivity of SLE patients is controversial. Skin tests, for instance, have been found to be either normal (28, 29) or lower than control values (30–34). The blastogenic response to phytohemagglutinin or to alloantigens has been found normal in some instances (28, 29, 31, 35, 36) and depressed in others (37–39). Values for percentage of peripheral T cells have been found highly variable from patient to patient (40–43).

It has been shown previously that the cytotoxic cells that are generated in a xenogeneic mixed lymphocyte reaction are of T origin, and recognize specifically H-2 K or H-2 D gene products (10, 16–18). Furthermore, we have recently observed (*a*) that the cytotoxic reaction was not inhibited by the presence in the medium of heat-aggregated immunoglobulins, nor by immune complexes and (*b*) that supernates from xenogeneic mixed leukocyte cultures were unable to induce cytotoxicity either per se or after the addition of naive PBL

TABLE III

*Response after 2-D Restimulation of Primed Human Lymphocytes*

| Experiment | Responder cells | Primary stimulation* | Secondary stimulation‡ | [3H]TdR uptake§ |
|---|---|---|---|---|
| | | | | *cpm ± SE* |
| 1 | Blood bank donor | none | none | 414±19 |
| | Blood bank donor | DBA/2(H-$2^d$) | none | 2,559±8 |
| | Blood bank donor | DBA/2(H-$2^d$) | C57BL/6 (H-$2^b$) | 3,459±286 |
| | Blood bank donor | DBA/2(H-$2^d$) | DBA/2 (H-$2^d$) | 12,210±3,144 |
| | SLE No. 12 | none | none | 580±96 |
| | SLE No. 12 | DBA/2(H-$2^d$) | none | 3,082±661 |
| | SLE No. 12 | DBA/2(H-$2^d$) | C57BL/6 (H-$2^b$) | 2,308±317 |
| | SLE No. 12 | DBA/2(H-$2^d$) | DBA/2 (H-$2^d$) | 18,191±779 |
| 2 | Blood bank donor | none | none | 251±16 |
| | Blood bank donor | DBA/2(H-$2^d$) | none | 2,651±66 |
| | Blood bank donor | DBA/2(H-$2^d$) | C57BL/6 (H-$2^b$) | 2,880±338 |
| | Blood bank donor | DBA/2(H-$2^d$) | DBA/2 (H-$2^d$) | 8,457±2,706 |
| | Urological patient | none | none | 670±46 |
| | Urological patient | DBA/2(H-$2^d$) | none | 2,890±1,046 |
| | Urological patient | DBA/2(H-$2^d$) | C57BL/6 (H-$2^b$) | 1,816±158 |
| | Urological patient | DBA/2(H-$2^d$) | DBA/2 (H-$2^d$) | 5,729±212 |
| | SLE No. 14 | none | none | 390±47 |
| | SLE No. 14 | DBA/2(H-$2^d$) | none | 5,916±776 |
| | SLE No. 14 | DBA/2(H-$2^d$) | C57BL/6 (H-$2^b$) | 2,756±499 |
| | SLE No. 14 | DBA/2(H-$2^d$) | DBA/2 (H-$2^d$) | 11,710±595 |

* $2 \times 10^7$ human PBL cocultured with $2 \times 10^7$ mitomycin C-treated mouse spleen cells for 9 d.

‡ $1 \times 10^5$ viable human PBL recovered at day 9, cocultured with $1 \times 10^6$ fresh spleen cells treated with mitomycin C.

§ Thymidine incorporation measured at day 2 of restimulation.

(data not shown). These arguments strongly argue against a cytotoxicity mechanism mediated by antibodies plus K cells. Thus the defect described in SLE patients bears either directly upon the cytotoxic T cells or their precursors, or indirectly upon cellular elements needed to support the differentiation of quiescent lymphocytes into killer cells. The presence of a normal MLC suggests that the defect does not involve a lack of helper activity. Several other mechanisms may be responsible for the T-cell functional alteration. A first possibility to be considered assumes a specific T-cell deficit. A dissociation between MLC and CML response has already been observed in the mouse after adult thymectomy or in aging mice (44, 45), two conditions in which the compartment of short lived (T1,Ly $123^+$) cells is selectively depleted. It is possible that such T1 lymphocytes are also lacking in lupus patients, a finding which would be in keeping with the selective loss of Ly $123^+$ cells in NZB mice (46), presenting a spontaneous lupus syndrome. Such an hypothesis would have the advantage of taking into account altogether an absence of killer cells after xeno-education, in spite of the occurrence of a normal MLC response. It would remain to be determined to what extent the selective loss of this T-cell subset would be linked to the pathogenesis of the disease and more precisely to the postulated defect in suppressor function described in NZB mice (47) and SLE patients (48).

An alternative hypothesis implies that the lack of functional cytotoxic T cells detected in SLE patients is a direct consequence of the presence of autoantibodies directed against lymphocytic antigens. Such autoantibodies have been detected by several groups in the serum of SLE patients (24, 51–55) as well as in the serum of NZB mice (49, 50). In a large survey of our patients (not including those of the present panel) it was found that 28 out of 39 had anti-lymphocytic autoantibodies.[2] The fine specificity of these antibodies is however still unclear. Some show relative restriction against T cells (24), whereas others are directed against both T and B lymphocytes (51); some seem to be restricted to "self" HLA (52, 53), others not (54, 55). In the light of the present results these autoantibodies would be

[2] F. Tron. Personal communication.

TABLE IV
*Effect of SLE Serum upon the Proliferative Response of PBL*

| Responder | SLE serum | | PHA response* | Allogeneic MLC‡ | | Xenogeneic MLC§ | |
|---|---|---|---|---|---|---|---|
| | | | | Control | Stimulated | Control | Stimulated |
| | | % | *cpm±SE* | | *cpm±SE* | *cpm±SE* | *cpm±SE* |
| Blood bank donor | —‖ | | 15,466±976 | 573±22 | 4,025±860 | 284±134 | 1,297±110 |
| | SLE No. 10¶ | 10 | 248±59 | | 2,716±380 | | 281±14 |
| | | 5 | 1,071±85 | | 2,550±354 | | 291±24 |
| | | 1 | 18,780±376 | | 3,488±216 | | 1,038±41 |
| | SLE No. 14¶ | 10 | 1,183±92 | | 2,125±584 | | 242±31 |
| | | 5 | 516±221 | | 3,558±74 | | 361±25 |
| | | 1 | 14,924±571 | | 6,169±168 | | 1,017±23 |
| SLE No. 10 | —‖ | | 24,433±677 | 545±140 | 3,850±13 | 279±51 | 1,321±12 |
| | SLE No. 10¶ | 10 | 1,520±114 | | 903±24 | | 139±3 |
| | | 5 | 3,003±23 | | 2,268±187 | | 994±44 |
| | | 1 | 20,534±670 | | 2,344±125 | | 3,060±48 |
| SLE No. 14 | —‖ | | 16,134±331 | 490±70 | 3,730±19 | 499±87 | 1,294±10 |
| | SLE No. 14¶ | 10 | 152±29 | | 2,027±52 | | 342±9 |
| | | 5 | 1,075±13 | | 2,481±100 | | 232±6 |
| | | 1 | 25,129±74 | | 4,734±64 | | 1,014±52 |

* PBL cultured with phytohemagglutinin (PHA)-M at a 1:100 dilution. Thymidine incorporation was measured at day 3.
‡ $1 \times 10^5$ PBL cocultured with $2 \times 10^5$ mitomycin-treated allogeneic lymphocytes. Thymidine incorporation was measured at day 6.
§ $1 \times 10^5$ PBL cocultured with $1 \times 10^6$ DBA/2 mouse spleen cells treated with mitomycin C. Thymidine incorporation was measured at day 4.
‖ Culture in medium supplemented with 20% AB serum.
¶ The culture medium was supplemented with 10% SLE serum + 10% AB serum, 5% SLE serum + 15% AB serum, and 1% SLE serum + 19% AB serum, respectively.

expected to be specifically directed against a T-cell subset involved in the generation or in the promotion of cytotoxic T cells. Antibodies capable of discriminating between subclasses of human T lymphocytes have been demonstrated in patients with juvenile rheumatoid arthritis (56). They may well be present in the serum of SLE patients too and this possibility is under investigation. The demonstration of blocking factors in the serum of SLE patients appears to provide support for the second type of interpretation. However the inhibition exerted by SLE serum in vitro affects both MLC and CML whereas the impairment observed with SLE lymphocytes cultured in normal AB serum is limited to the expression of cytotoxic T cells.

One might hypothesize that the same serum inhibitors whose activity is demonstrated in vitro, already impair in vivo the potential reactivity of PBL from SLE patients. However, this hypothesis is not favored by the finding that SLE serum can further inhibit the proliferative response of autologous SLE lymphocytes when added in vitro to the culture medium although these lymphocytes were already in contact with the same serum in vivo, and the observation that the preincubation of normal donor PBL with SLE serum for 2 h at 37°C does not lead to the inactivation of these lymphocytes when they are later educated in normal AB serum. Thus the in vitro inhibition of lymphocyte responses by SLE serum factors, is probably not a simple mimicry of what occurs in vivo on the PBL of SLE patients. This conclusion does not preclude however the possibility that the in vivo immune deficit might have a humoral origin.

Some of the SLE sera tested for in vitro inhibition of MLC and CML contained high amounts of immune complexes. Others did not, but this may just have reflected a relative lack of sensitivity of the detecting assay. Some T-cell subsets and, in particular, activated T lymphocytes (57) express Fc receptors and might therefore fix complexes. It remains to be determined however to what extent the occupation of Fc receptors by immune complexes may modify the functions of educated T cells. One should note, in that regard, that the shedding of Fc receptors at 37°C has no repercussion upon the cytolytic capacity of educated T lymphocytes (58).

In conclusion, two salient features emerge from this study on the immune responsiveness of SLE patients. One is the incapacity of such patients' T lymphocytes

TABLE V

*Effect of SLE Serum During or before Education upon the Differentiation of Normal Donor PBL into Cytolytic T Cells*

| Experiment | Serum* source | Percent specific chromium release‡ effector:target cell ratio | | | | Xenogeneic MLC§ | |
|---|---|---|---|---|---|---|---|
| | | 100:1 | 50:1 | 25:1 | 12:1 | Control | Stimulated |
| | | % | | | | *cpm*±*SE* | |
| 1 | AB | 38 | 36 | 23 | 13 | 355±72 | 16,869±309 |
| | SLE 3‖ | 0 | 2 | 1 | 0 | 431±134 | 4,734±117 |
| 2 | AB | 44 | 31 | 24 | 19 | 499±99 | 8,670±736 |
| | SLE 16‖ | 5 | 0 | 0 | 0 | 573±229 | 1,171±430 |
| 3 | AB | 46 | 35 | 24 | 15 | | |
| | SLE 17‖ | 10 | 9 | 6 | 3 | ND | |
| | SLE 17 preincubation¶ | 50 | 43 | 34 | 11 | | |

* Cultures were performed in medium supplemented with 20% heat-inactivated AB serum.

‡ PBL educated against DBA/2 spleen cells and tested for cytotoxicity at day 7 against $^{51}$Cr-labeled P 815 target cells.

§ $1 \times 10^5$ PBL cocultured with $1 \times 10^6$ DBA/2 mouse spleen cells treated with mitomycin C. Thymidine incorporation was measured at day 4.

‖ In those cultures the medium contained a mixture of 10% SLE serum and 10% AB serum.

¶ PBL were first incubated at 37°C for 2 h in 10% SLE serum, then washed twice and finally resuspended in medium enriched with 20% AB serum.

to express significant cytotoxicity against xenogeneic target cells, in spite of a normal proliferative response in MLC. The other is the inhibitory effect of their serum on T-cell proliferation and generation of cytolytic cells. It is at present difficult to decide whether these two observations are independent manifestations of the same disease or whether they are related in a way that remains to be determined? No simple experiment can provide a definitive answer to such a basic question posed for all studies of cellular parameters in human lupus. This important reservation does not detract interest from the whole problem of evaluating T cell-mediated immunity in human or murine lupus but urges for caution in interpreting the results in terms of the pathogenesis of the disease.

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## REFERENCES

1. Kunkel, H. G., and E. M. Tan. 1964. Autoantibodies and disease. *Adv. Immunol.* **4:** 351–359.
2. Allison, A. C., A. M. Denman, and R. D. Barnes. 1971. Cooperating and controlling function of thymus-derived lymphocytes in relation to autoimmunity. *Lancet.* **I:** 135–139.
3. Holman, H. R. 1971. Systemic lupus erythematosus. Immunological disease. M. Sampter, editor. Little Brown & Company, Boston, Mass. 2nd edition. 995–1014.
4. Cochrane, C. G., and D. Koffler. 1973. Immune complex disease in experimental animals and man. *Adv. Immunol.* **16:** 185–264.
5. Quismorio, F. P., and G. J. Friou. 1974. Immunologic phenomena in patients with systemic lupus erythematosus. *In* Lupus Erythematosus. E. L. Dubois, editor. University of Southern California Press, Los Angeles, Calif. 2nd edition. 164–190.
6. Krishnan, C., and M. H. Kaplan. 1967. Immunopathologic studies of systemic lupus erythematosus. *J. Clin. Invest.* **46:** 569–579.
7. Harbeck, R. J., E. J. Bardana, P. T. Kohler, and R. J. Carr. 1973. DNA anti-DNA complexes: their detection in systemic lupus erythematosus. *J. Clin. Invest.* **52:** 789–795.
8. Talal, N. 1970. Immunologic and viral factors in the pathogenesis of systemic lupus erythematosus. *Arthritis Rheum.* **13:** 887–894.
9. Talal, N., and A. D. Steinberg. 1974. The pathogenesis of autoimmunity in New Zealand Black Mice. *Curr. Top. Microbiol. Immunol.* **64:** 79–103.
10. Carnaud, C., M. Fadai-Ghotbi, P. Lesavre, and J. F. Bach. 1977. Education of human lymphocytes against mouse cells: specific recognition of H-2 antigens. *Eur. J. Immunol.* **7:** 81–85.
11. 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:** 643–648.
12. Bach, J. F. 1973. Evaluation of T cells and thymic serum factors in man using the rosette technique. *Transplant. Rev.* **16:** 196–217.
13. Bianco, C., R. Patrick, and V. Nussenzweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen antibody complement complexes. *J. Exp. Med.* **132:** 702–720.
14. Pincus, T., P. H. Schur, J. A. Rose, and N. Talal. 1969. Serum DNA binding in systemic lupus erythematosus. *N. Engl. J. Med.* **281:** 701–705.
15. Digeon, M., M. Laver, J. Riza, and J. F. Bach. 1977. Detection of circulating immune complexes in human sera by simplified assays with polyethylene glycol. *J. Immunol. Methods.* **16:** 165–183.
16. Lindhal, K. F., and F. H. Bach. 1975. Human lymphocytes recognize mouse alloantigens. *Nature (Lond.).* **254:** 607–609.
17. Widmer, M. B., and F. H. Bach. 1972. Allogeneic and xenogeneic response in mixed leucocyte cultures. *J. Exp. Med.* **135:** 1204–1208.
18. Lindhal, K. F., and F. H. Bach. 1976. Genetic and cellular aspects of xenogeneic mixed leucocyte culture reaction. *J. Exp. Med.* **144:** 305–318.
19. Cousar, J. B., and D. A. Horwitz. 1973. Inhibition of lymphocyte activation by sera of patients with systemic lupus erythematosus. *Arthritis Rheum.* **16:** 539.
20. Wernet, P., M. Fotino, R. Thoburn, A. Moore, and H. G. Kunkel. 1973. Blockage of lymphocyte surface antigens and the shedding phenomenon in systemic lupus erythematosus. *Arthritis Rheum.* **16:** 137–138.
21. Siegal, F. P., P. Wernet, R. Thoburn, Z. Bentwich, R. J. Winchester, and H. G. Kunkel. 1974. Anti-lymphocytes antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **17:** 501.
22. Williams, R. C., Jr., R. B. Lies, and R. P. Messner. 1973. Inhibition of mixed leucocyte culture responses by serum and gamma globulin fractions from certain patients with connective tissue disorders. *Arthritis Rheum.* **16:** 597–605.
23. Stastny, P., and M. Ziff. 1971. Antibodies against cell membrane constituents in systemic lupus erythematosus and related diseases. *Clin. Exp. Immunol.* **8:** 543–550.
24. Lies, R. B., R. P. Messner, and R. C. Williams, Jr. 1973. Relative T-cell specificity of lymphocytotoxins from patients with systemic lupus erythematosus. *Arthritis Rheum.* **16:** 369–375.
25. Paty, J. G., C. W. Sienknecht, A. S. Townes, A. S. Hanissian, J. B. Miller, and A. T. Masi. 1975. Impaired cell-mediated immunity in systemic lupus erythematosus. *Am. J. Med.* **59:** 769–779.
26. Horwitz, D. A., and J. B. Cousar. 1975. A relationship between impaired cellular immunity, humoral suppression of lymphocyte function and severity of lupus erythematosus. *Am. J. Med.* **58:** 829–835.
27. Utsinger, P. D. 1976. Lymphocyte responsiveness in systemic lupus erythematosus. *Arthritis Rheum.* **19:** 88–92.
28. Foad, B., S. Khullar, E. Freimer, A. Kissner, and R. Sheon. 1975. Cell mediated immunity in systemic lupus erythematosus. Alterations with advancing age. *J. Lab. Clin. Med.* **85:** 132–139.
29. Goldman, J., H. Litwin, L. E. Adams, R. C. Krueger, and E. V. Hess. 1972. Cellular immunity in systemic lupus erythematosus. *J. Clin. Invest.* **51:** 2669–2677.
30. Bitter, Th., F. Bitter, R. Silberschmidt, and E. L. Dubois. 1971. In vivo and in vitro study of cell-mediated immunity during the onset of systemic lupus erythematosus. *Arthritis Rheum.* **14:** 152–153.
31. Horwitz, D. A. 1972. Impaired delayed hypersensitivity in systemic lupus erythematosus. *Arthritis Rheum.* **15:** 353–360.
32. Toh, B. H., I. C. Roberts-Thomson, J. D. Mathews, S. Whittingham, and I. R. Mac Kay. 1973. Depression of cell-mediated immunity in old age and the immunopathic diseases, lupus erythematosus, chronic hepatitis, and rheumatoid arthritis. *Clin. Exp. Immunol.* **14:** 193–202.
33. Block, S., Ch. Gibbs, M. Betty-Stevens, and L. Shulman. 1968. Delayed hypersensitivity in systemic lupus erythematosus. *Ann. Rheum. Dis.* **27:** 311–318.
34. Hahn, B., M. Bagby, and K. Osterland. 1973. Abnormalities of delayed hypersensitivity in systemic lupus erythematosus. *Am. J. Med.* **55:** 25–32.
35. Patrucco, A., N. F. Rothfield, and K. Hirschorn. 1967. The response of cultured lymphocytes from patients with systemic lupus erythematosus to DNA. *Arthritis Rheum.* **10:** 32–33.
36. Senyk, G., W. K. Hadley, M. R. Attias, and N. Talal. 1974. Cellular immunity in systemic lupus erythematosus. *Arthritis Rheum.* **17:** 553–562.
37. Suciu-Foca, N., J. A. Buda, T. Thiem, and K. Reemtsma. 1974. Impaired responsiveness of lymphocytes in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **18:** 295–301.
38. Malave, L., Z. Layrisse, and M. Layrisse. 1975. Dose dependent hyporeactivity to phytohemagglutinin in systemic lupus erythematosus. *Cell. Immunol.* **15:** 231–236.
39. Utsinger, P. D., and W. J. Yount. 1977. Phytohemagglutinin response in systemic lupus erythematosus. *J. Clin. Invest.* **60:** 626–638.
40. Messner, R. P., F. D. Lindstrom, and R. C. Williams, Jr. 1973. Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosus. *J. Clin. Invest.* **52:** 3046–3056.
41. Scheinberg, M. A., and E. S. Cathcart. 1973. B and T cell lymphopenia in SLE. *Arthritis Rheum.* **16:** 566–567.
42. Scheinberg, M. A., and E. S. Cathcart. 1974. B cell and T cell lymphopenia in systemic lupus erythematosus. *Cell. Immunol.* **12:** 309–319.
43. Williams, R. C., Jr., J. K. Deboard, O. J. Mellbye, R. P. Messner, and F. K. Lindström. 1973. Studies of T and B lymphocytes in patients with connective tissue diseases. *J. Clin. Invest.* **52:** 283–295.
44. Bach, M. A. 1977. Lymphocyte mediated cytotoxicity: effect of ageing, adult thymectomy and thymic factor. *J. Immunol.* **119:** 641–647.
45. Andersson, L. C., P. Hayry, M. A. Bach, and J. F. Bach. 1974. Difference in the effect of adult thymectomy in T-cell mediated responses in vitro. *Nature (Lond.).* **252:** 252.
46. Cantor, H., L. Mac Vay-Boudreau, J. Hugenberger, K. Naidorf, F. N. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T cell sets. II. Physiologic role of feed back inhibition in vivo: absence in NZB mice. *J. Exp. Med.* **147:** 1116–1125.
47. Gerber, N. L., J. A. Hardin, T. M. Chused, and A. D. Steinberg. 1974. Loss with age in NZB/W mice of thymic suppressor cells in the graft-versus-host reaction. *J. Immunol.* **113:** 1618–1625.
48. Breshnihan, B., and H. E. Jasin. 1977. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. Clin. Invest.* **59:** 106–116.
49. Shirai, T., and R. C. Mellors. 1971. Natural thymocytotoxic

autoantibody and reactive antigen in New Zealand Black and other mice. *Proc. Natl. Acad. Sci. U. S. A.* **68:** 1412.
50. Parker, L. M., T. M. Chused, and A. D. Steinberg. 1974. Immunofluorescence studies on thymocytotoxic antibody from New Zealand Black mice. *J. Immunol.* **112:** 285–292.
51. Winchester, R., J. B. Winfield, Shu Man Fu, and H. Kunkel. 1975. Studies on antilymphocyte antibodies in patients with rheumatoid arthritis and systemic lupus erythematosus. *Rheumatology.* **6:** 20.
52. Mittal, K., R. Rossen, J. Sharp, M. Lidsky, and W. Butler. 1970. Lymphocyte cytotoxic antibodies in systemic lupus erythematosus. *Nature (Lond.).* **225:** 1255–1256.
53. Terasaki, P., V. Mottironi, and E. Barnett. 1970. Cytotoxins in disease. Autocytotoxins in lupus. *N. Engl. J. Med.* **283:** 724–728.
54. Butler, W., J. Sharp, R. Rossen, M. Lidsky, K. Mittal, and D. Gard. 1972. Relationship of the clinical course of systemic lupus erythematosus to the presence of circulating lymphocytotoxic antibodies. *Arthritis Rheum.* **15:** 231.
55. Nies, K., J. Brown, E. Dubois, F. Quismorio, G. Friou, and P. Terasaki. 1974. Histocompatibility (HL-A) antigens and lymphocytotoxic antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **17:** 397.
56. Schlosman, S. F., R. L. Evans, and A. J. Strelkauskas. 1978. Human T cell subsets with regulatory functions. *In* Human Lymphocyte Differentiation: Its Application to Cancer. B. Serrou and C. Rosenfeld, editors. Elsevier/North Holland, Amsterdam. 83–92.
57. Stout, R. D., S. D. Waksal, and L. A. Herzenberg. 1976. The Fc receptor on thymus derived lymphocytes. III. Mixed lymphocyte reactivity and cell-mediated lympholytic activity of Fc− and Fc+ T lymphocytes. *J. Exp. Med.* **144:** 54–68.
58. Leclerc, J. C., C. Plater, and W. H. Fridman. 1977. The role of the Fc receptor (FcR) of thymus derived lymphocytes. 1. Presence of FcR on cytotoxic lymphocytes and absence of direct role in cytotoxicity. *Eur. J. Immunol.* **8:** 543.