Antibody-Dependent Cell-Mediated Cytotoxicity in Selected Autoimmune Diseases

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A B S T R A C T Antibody-dependent cell-mediated cytotoxicity mediated by peripheral blood lymphocytes was studied in patients with systemic lupus erythematosus, polyarteritis nodosa, Sjogren's syndrome, and rheumatoid arthritis. The target cells were chicken erythrocytes coated with rabbit anti-chicken erythrocyte antibody. Antibody-dependent cell-mediated cytotoxic activity was normal in Sjogren's syndrome and rheumatoid arthritis but significantly decreased ($P < 0.001$) in active systemic lupus erythematosus and in two patients with polyarteritis nodosa.

A partial regeneration of antibody-dependent cell-mediated cytotoxic activity was obtained by treatment with pronase and DNase followed by overnight incubation. Sera from patients with systemic lupus erythematosus inhibited antibody-dependent cell-mediated cytotoxic activity of normal lymphocytes. The inhibitory activity was studied by specific immunoadsorption and sucrose density gradient ultracentrifugation. Removal of IgG but not IgM greatly reduced inhibition. Inhibitory factors were present in 7S and heavier fractions containing IgG.

Five systemic lupus erythematosus patients were studied serially to determine if improvement in clinical status could be correlated with a decrease in serum inhibitory factors as studied by inhibition of normal antibody-dependent cell-mediated cytotoxicity. Indeed, a greater serum inhibitory capacity was found in each patient during periods of greater disease activity.

INTRODUCTION

Human peripheral blood lymphoid cells are a heterogenous population consisting of T cells which mediate cellular immunity, B cells which mediate humoral immunity, and a third cell population defined by its ability to lyse antibody-coated target cells (1, 2). This third population, generally called K or killer cells, may cause tissue destruction by the mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC).[1] ADCC has been implicated as an important immunologic defense mechanism against malignancy (3). Its involvement in renal transplant rejection (4) and in autoimmune tissue destruction (5, 6) has been postulated. Furthermore, ADCC has been proposed as a way of inducing immunologic tolerance, particularly in newborns (7).

A variety of effector cells mediate ADCC, including monocytes and polymorphonuclear leukocytes (8). K cells have the morphological appearance of small lymphocytes but lack the surface markers normally associated with mature T and B cells. They possess receptors for the Fc portion of IgG and for C_3 (1). Lysis is dependent on the binding to this Fc receptor of specific antibody attached to target cells. K cells that bind immunoglobulin or circulating immune complexes in vivo can be incor-

[1] Abbreviations used in this paper: ADCC, antibody-dependent cell-mediated cytotoxicity; CRBC, chicken erythrocytes; FCS, fetal calf serum; MEM, minimal essential medium; PAN, polyarteritis nodosa; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; TCM, tissue culture medium; TH, tris-buffered Hank's salt solution.

rectly identified as B lymphocytes on the basis of membrane immunofluorescence with anti-immunoglobulin sera (9).

ADCC activity may be abnormal in patients with autoimmune and lymphoproliferative diseases. Recently, patients with systemic lupus erythematosus (SLE) were reported to have decreased ADCC activity against antibody-coated Chang cells (10). In the present report, we investigated ADCC activity against antibody-coated chicken erythrocytes (CRBC) by peripheral blood lymphocytes in patients with SLE, polyarteritis nodosa (PAN), Sjorgren's syndrome (SS) and rheumatoid arthritis (RA). Compared to normal controls, activity was decreased in SLE and PAN but not in SS and RA. Decreased activity was partially related to serum inhibitory factors present in SLE patients.

METHODS

Patient population. 15 patients (11 females and 4 males) with SLE, 15 patients (12 females and 3 males) with SS, 7 patients (all males) with RA, 2 patients with PAN and 22 normal controls (11 females and 11 males) were studied. The mean age for the controls was 37 yr (range 25–59).

The patients with SLE had at least four of the preliminary criteria proposed by the Arthritis and Rheumatism Association for the diagnosis of SLE (11). Their mean age was 37 yr (range 17–59). 11 patients had active and 4 inactive disease. SLE was considered active when clinical symptoms (e.g., arthritis, nephritis, serositis, cerebritis, or widespread cutaneous lesions) were associated with two or more of the following laboratory parameters: Westergren sedimentation rate > 20/h, lymphopenia < 1,000/mm^3, serum complement level decreased (C3 < 55 mg/ 100 ml, CH^{50} < 25 hemolytic units), DNA binding > 20% (filter radioimmunoassay). All active SLE patients were receiving corticosteroids (10–80 mg/day, mean 32 mg). Most inactive SLE patients also received corticosteroids (0–30 mg/day, mean 14 mg). One patient was receiving chlorambucil (4 mg/day).

The patients with SS fulfilled at least two of the following criteria: xerostomia with decreased parotid salivary flow, keratoconjunctivitis sicca with abnormal Schirmer test and Rose Bengal staining, and an associated connective tissue disease. Their mean age was 52 (range 21–77). The diagnosis was confirmed in all cased by a lip biopsy showing significant focal lymphoid infiltration (more than one focus of 50 mononuclear cells/4 mm^2 of tissue). Six patients had associated RA, and one each had scleroderma, inactive SLE, or primary biliary cirrhosis.

The 7 RA patients fulfilled the Arthritis and Rheumatism Association criteria for a definite diagnosis. Mean age of these patients was 53 (range 45–71). Two patients were on corticosteroids (9 and 20 mg/day). The remaining patients were treated symptomatically.

Diagnosis of PAN was based on the presence of biopsy proven necretizing perivascular infiltration and angiographic demonstration of dilatations in the small to medium sized arteries in the visceral circulation.

Materials. Eagles minimal essential medium (MEM) with 25 mM Hepes buffer (Gibco, New York) supplemented with 5% heat decomplemented fetal calf sera, (FCS), antibiotic-antimycotic, and 2 mM glutamine was used through-

out as the tissue culture medium (TCM). Hanks' balanced salt solution made to 0.05 M, pH 7.4 with Tris-HCl buffer (TH) and containing 2.5% heat decomplemented FCS (TH-FCS) was employed for in vitro manipulation of CRBC. Sodium-Chromate-51 (200–500 Ci/g) was purchased from New England Nuclear (Boston, Mass.). Goat anti-human IgG and IgM were obtained from Meloy Laboratories Inc., (Springfield, Va.).

Isolation and purification of peripheral blood mononuclear cells. The effector cells for the ADCC assay were isolated from heparinized human peripheral blood. Mononuclear cells were obtained by flotation sedimentation using a Ficoll-Hypaque gradient according to Boyum (12). After this treatment, phagocytic cells were removed by incubation with Technicon lymphocyte separating reagent (containing carbonyl iron coated with poly-L-lysine) at 37°C for 30 min before passage through a magnetic field (Technicon Lymphocyte Separator, Technicon Inst. Corp., Tarrytown, N. Y.).

The resulting cell population contained more than 98% lymphocytes (Wright staining), with greater than 95% viability as judged by trypan blue dye exclusion. The cells were washed three times in TCM before use. Cell counts were made with a Coulter Counter Model ZB1 (Coulter Electronics Inc., Hialeah, Fla.). To assure accuracy, automatic counts were compared frequently with optical counts determined in a hemocytometer.

Preparation of antiserum. CRBC freshly obtained from a white leghorn chicken were placed in TH containing heparin. The cells were washed 3 times with TH-FCS and resuspended as a 10% suspension in saline. A New Zealand White rabbit was immunized intravenously with 1 ml of the 10% CRBC suspension biweekly for 3 wk. After a 2 mo rest, the rabbit was again immunized one time with 1 ml CRBC and bled by cardiac puncture 1 wk later. The anti-CRBC sera was decomplemented by heat inactivation (56°C for 30 min), aliquoted, and stored at −90°C until used.

ADCC. ADCC was measured by the procedure of Larsson et al. (13), with modification.

For labeling with Na$_2$Cr^{51}O$_4$, CRBC were freshly obtained and washed as above. The cells were resuspended to a concentration of 1.5 × 10^8/ml in TH-FCS. 0.2 ml of a 1 mC/ml solution of Na$_2$Cr^{51}O$_4$ was mixed with 0.2 ml of TH-FCS. 0.2 ml of the above CRBC suspension was then added and the mixture incubated for 1 h at 37°C. The labeled cells were washed 3 times with TH-FCS, resuspended in 3 ml of the same buffer, and counted.

CRBC were diluted into TCM containing a 10^{-4} dilution of rabbit anti-CRBC (for experimental group) or no antisera (control group) to yield 8 × 10^5 labeled CRBC/ml. Cultures were performed in quadruplicate in microtiter plates (Limbro ISMRO-96TC, Limbro Chemical Co., New Haven, Conn.). The isolated mononuclear cells were employed as effector cells. First, 50 μl of TCM containing either 3.2 × 10^6, 1.6 × 10^6, or 8 × 10^4 mononuclear cells (representing effector to target cell ratios of 4, 2, and 1:1, respectively) were added to the wells by automatic pipette. This was followed by 100 μl of labeled CRBC. Cultures were incubated at 37°C for 4 h in a humid atmosphere containing 5% CO$_2$. Assays were performed by centrifuging the microplates at 250 g for 5 min and withdrawing 75 μl of supernate from each well. The supernate was placed in 10 × 75-mm tubes, made up to 1 cc with distilled water and radioactivity was determined in a Packard Gamma Scintillation Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). To determine maximum release (cpm$_{max}$), complement dependent lysis was assayed by

adding 50 μl normal human serum, as a source of complement, to 100 μl antibody coated CRBC. Spontaneous release controls consisted of lymphocytes plus labeled CRBC without antiserum (cpm_spont.). The percent cytotoxicity was calculated on a Wang 700 programmable calculator (Wang Laboratories, Inc., Tewksbury, Mass.) using the following formula:

$$\frac{CPM_{exp} - CPM_{spont}}{CPM_{max} - CPM_{spont}}$$

Regeneration of ADCC activity by overnight incubation. ADCC was performed on patient and normal PB lymphocytes as above. A portion of the purified lymphocytes was saved and the cells resuspended in MEM without FCS to a concentration of 6.4×10^6 cells/ml. 100 μg of DNase was added to 1-ml portions of lymphocytes to prevent clumping (14). Pronase at a concentration of 10 μg/ml was then added to some samples. (It was found that in some but not all cases the addition of 10 μg/ml pronase could augment the regeneration after washings and overnight incubation). The cells were then incubated at 37°C for 1 h in a humid atmosphere containing 5% CO_2. The cells were washed 3 times in TCM, resuspended in 1 ml TCM, and incubated overnight at 37°C. After the incubation, cells were again washed 3 times and the ADCC activity examined.

Inhibition of normal ADCC activity by serum or serum fractions. Sera from patients and normals were heat inactivated at 56°C for 30 min. Some sera were fractionated on a 10–35% sucrose gradient in borate-buffered saline (0.15 M, pH 8.0) (15). 0.2 ml of heat inactivated sera was layered on 13 ml of the gradient and the gradients were centrifuged at 4°C at 39,000 rpm in a SW 40 rotor for 20 h using a Beckman Model L265 B ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). 350-μl fractions were collected from the gradient. Localization of the IgM (19S) and IgG (7S) peaks was performed by radial immunodiffusion using immunoplates (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.). The sucrose gradients were pooled and dialyzed against MEM.

Selected sera were depleted of IgG or IgM with Sepharose 4B immunoadsorbents. Globulin fractions from goat antihuman IgG, antihuman IgM or normal goat sera, were prepared by precipitation with 45% ammonium sulphate. After dialysis (0.1 M sodium bicarbonate, 0.5 M sodium chloride) the fractions were coupled to CNBr-activated Sepharose 4-B (no. 74301 Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) according to the manufacturer. Protein concentrations were calculated using $E_{1\%\ 280\ nM} = 14.0$. Coupling in all cases was greater than 95% yielding approximately 15 mg protein/g activated Sepharose (dry weight).

For adsorption, 0.2 ml serum was diluted to 1.5 ml in phosphate buffered saline (0.01 M phosphate, 0.15 M sodium chloride, pH 7.4) and added to an equal volume of immunoadsorbent. The suspension was rotated for 2 h at room temperature and the adsorption repeated with a fresh sample of immunoadsorbent. After adsorption, the samples were dialyzed against TCM. The efficiency of absorption was shown by the absence of IgG or IgM in appropriately adsorbed samples as determined by radial immunodiffusion.

For inhibition experiments we modified TCM by replacing the 5% FCS with 0.5% bovine serum albumin. Normal lymphocytes were adjusted to 6.4×10^6 cells/ml in TCM, an equal volume of heat decomplemented normal or patients sera, whole or fractionated, was added and the mixture preincubated at 37°C for 1 h. Gradient fractions

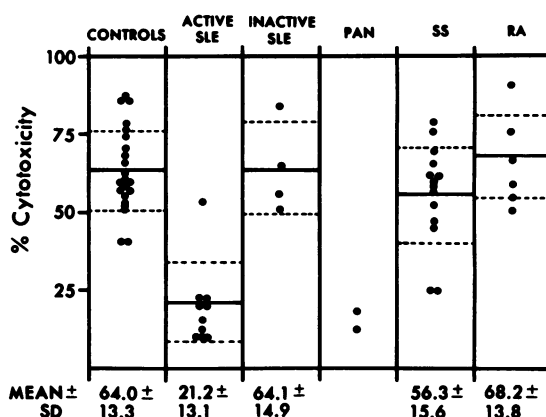## ADCC Activity in Controls and in Patients

FIGURE 1 ADCC activity of lymphocytes from patients and controls at an effector to target ratio of 2:1. Solid line represents mean and dashed lines the standard deviation in each group.

were studied at comparable IgG concentrations as determined by radial immunodiffusion. 50 μl of the above mixture was then added to the microtiter plates and 100 μl of a chromium⁵¹ labeled CRBC mixture added. The mixture was the same as in the regular ADCC experiment with the exception that TCM for inhibition was used and the antisera in the experimental group was at a 5×10^{-5} dilution instead of 10^{-4}. The culture and termination conditions were the same as previously described.

RESULTS

The ADCC activity of purified mononuclear cells from healthy controls is shown in Fig. 1. At an effector to target cell ratio of 2:1, the mean cytotoxicity was $64.0 \pm 13.3\%$. In patients with active SLE, a significantly lower ADCC activity ($21.2 \pm 13.1\%$) was found compared to normals ($P < 0.001$). In patients with inactive SLE, the ADCC activity ($64.1 \pm 14.5\%$) was not significantly different from the normal controls. Two patients with PAN resembled the SLE patients and showed less than 20% cytotoxicity.

The ADCC activity in patients with SS and patients with seropositive RA did not differ significantly from controls.

Regeneration of ADCC activity. The impaired ADCC activity in SLE and PAN patients could be due to cell-bound blocking factors directly occupying or sterically interfering with the Fc receptors on K cells. To test this hypothesis, lymphocytes from patients and normal controls were examined in the ADCC assay both directly after isolation or after exposure to pronase and overnight incubation at 37°C. This treatment is known to eliminate immunoglobulins and blocking factors attached to SLE lymphocytes (16). In SLE, pronase and overnight incubation increased ADCC activity by

TABLE I

ADCC Activity Before and After Treatment with Pronase and Overnight Incubation

Patient	Before treatment	After treatment	Cytotoxicity*
			Δ%
SLE‡			
1	34.8	53.7	18.9
2	15.4	30.0	14.7
3	22.3	40.0	17.7
4	20.4	33.4	13.0
5	10.2	19.4	9.2
	Mean = 20.6±9.2	= 35.3±12.7	= 14.7±3.9
PAN			
1	19.9 (Expt. 1)	53.4	33.5
	12.7 (Expt. 2)	41.3	28.6
2	17.7	68.5	50.8
	Mean = 16.7±3.7	= 54.5±13.7	= 37.6±11.7
Normal controls			
1	60.7	69.3	8.6
2	69.5	67.7	0
3	61.0	63.6	2.6
4	87.3	89.6	2.3
	Mean = 69.6±12.5	= 72.5±11.6	= 3.4±3.7

* Δ percent cytotoxicity = percent cytotoxicity in ADCC after treatment, percent cytotoxicity in ADCC before treatment.

‡ SLE compared to normal controls: Δ percent cytotoxicity $P < 0.02$.

$14.7±3.9\%$ whereas the increase in normal control lymphocytes was only $3.4±3.7\%$ (Table I). Moreover, even after this treatment, the mean percent cytotoxicity in SLE ($35.3±12.7$) was still well below the mean of normal lymphocytes after pronase and overnight incubation ($72.5±11.6\%$, $P < 0.01$). In the PAN patients, the lymphocytes showed a more complete regeneration after treatment, with a mean increase in ADCC activity of $37.6±11.7\%$. The ADCC activity in PAN after treatment ($54.5±13.7\%$) did not differ significantly from controls ($72.5±11.6\%$, $P > 0.05$).

Inhibition of ADCC by SLE sera. To evaluate the source of possible cell-bound blocking factors, we examined SLE sera for ability to inhibit the ADCC activity of normal human lymphocytes. Normal lymphocytes were preincubated for 1 h at 37°C in heat decomplemented human serum. When autologous or random normal serum was studied, less than 15% inhibition was observed (compared to the same lymphocytes in medium alone).

When sera from patients with active lupus were studied, the degree of inhibition of ADCC activity of normal lymphocytes ranged from 0–75% with a mean of $45.7±25\%$ (Table II). The sera of all SLE patients with significantly decreased ADCC showed blocking

capability. Sera from a patient with PAN also showed inhibition of normal ADCC function. In this patient, the inhibitory capacity of the sera correlated inversely with the ADCC activity of the patient's lymphocytes.[a]

Characterization of serum inhibitory factors. To determine whether immunoglobulins were responsible for the observed serum inhibition of normal ADCC, we selectively removed IgG or IgM from the sera of two SLE patients and one normal control by specific immunoadsorption. Removal of IgG from two SLE sera by incubation with Sepharose-goat anti-human IgG (Table III) resulted in 16.3 and 7.3% inhibition, respectively. By contrast, removal of IgM by immunoadsorption resulted in a much higher inhibition (60.4 and 65.0%) that was similar to a control incubation with a normal goat globulin absorbent.

Sera from SLE patients and normal controls were fractionated on 10–35% linear sucrose density gradients. Gradients were pooled into three major fractions representing 7S, 19S, and greater than 19S regions. The pooled fractions were dialysed against TCM and tested for their ability to inhibit normal lymphocytes in ADCC (Table IV). In the five SLE patients tested, the 7S

[a] Fye, K., M. Becker, A. Theofilopoulos, H. Moutsopoulos, J. L. Feldmann, and N. Talal.

TABLE II
Inhibition of ADCC Activity of Normal Lymphocytes by Sera from SLE Patients

Patient	Inhibition of normal ADCC* by sera	ADCC of patients' lymphocytes (cytotoxicity)‡
	%	%
1	0	54
2	25	21
3	29	20
4	34	12
5	48	10
6	65	35
7	65	22
8	70	20
9	75	15

* See Methods for procedure. Percent inhibition calculated using normal sera as 0% inhibition base line.
‡ Percent cytotoxicity at a 2:1 effector to target ratio.

gradient fractions were inhibitory compared to normal 7S fractions prepared in the same way and studied at similar concentrations. In two SLE patients studied, the 19S and greater than 19S fractions were also inhibitory compared to comparable fractions from normal sera. These heavier fractions were found to contain IgG, possibly in immune complexes capable of binding to the Fc receptors of K cells.

Serum inhibitory factors and disease activity. Sera from individual SLE patients were studied serially to determine if a decrease in inhibitory capacity correlated with improvement in the clinical status. In each of five SLE patients studied, a greater inhibitory capacity was associated with the more active disease state (Table V).

DISCUSSION

This study has demonstrated a significant decrease in ADCC activity in patients with active SLE, related in part to inhibitory serum factors. Two patients with PAN

TABLE III
Inhibition of Normal ADCC after Removal of IgG or IgM by Specific Immunoadsorption of SLE Sera

Patient	Inhibition after immunoadsorption with:		
	anti-IgG	anti-IgM	normal goat globulin
		%	
1	16.3*	60.4	ND
2	7.3	65.0	55.6

ND, not done.
* Percent inhibition calculated by using immunoadsorbent passed normal sera as 0% inhibition base line.

TABLE IV
Inhibition of ADCC Mediated by Normal Lymphocytes with Sucrose Gradient Fractions from SLE Patients

Patient	Inhibition*		
	7S	19S	>19S
		%	
1	36	33‡	39‡
2	52	41‡	33‡
3	44	ND	ND
4	19	ND	ND
5	20	ND	ND

ND, not done.
* Inhibition calculated by using comparable gradient fractions from normal sera at same IgG concentration for 0% inhibition.
‡ IgG detected by radial immunodiffusion.

also displayed reduced ADCC, while this activity in SS and RA patients was similar to that of normal individuals. A recent publication also finds ADCC to be normal in RA (17).

A significant increase in the ADCC capability of SLE lymphocytes was achieved by exposure of the cells to pronase followed by overnight incubation at 37°C and repeated washings. This partial regeneration of ADCC activity in SLE suggests that cell-bound blocking factors contribute to the decreased K cell function. Other authors have shown that incubation of SLE lymphocytes results in the release on inhibitory factors and restoration of mixed lymphocyte reactivity (18). The inability to achieve complete restoration of ADCC activity in

TABLE V
Change in Serum Inhibitory Activity of SLE Patients' Sera against Normal Lymphocytes as a Function of Disease Status

SLE patient	Inhibition*		Therapy§
	Low complement‡ (active disease)	Normal complement	
	%		
1	54	6	Chlorambucil-prednisone
2	50	0	None
3	63	48	Prednisone
4	45	27	Aspirin
5	87	58	None

* See Methods. Percent inhibition calculated using normal sera as 0% inhibition base line.
‡ Low complement levels were associated with elevated titers of anti-double stranded-DNA antibody.
§ Therapy during active disease.

SLE may indicate an additional factor contributing to this defect, possibly a decrease in the number or function of K cells themselves. A quantitative defect in K cells could result from the action of cytotoxic anti-K cell antibodies or could reflect sequestration of K cells in parenchymal organs. A precedent for the former mechanism is the reduction in the absolute number of T cells in SLE which may be due to anti-T cell antibodies (19).

The more complete regeneration of ADCC function in a limited number of PAN patients suggests that the functional capacity of K cells themselves may be less impaired in this disease. Decrease in ADCC activity in PAN is probably due to blocking of Fc on K cells by immune complexes known to occur in some patients with PAN (20). Indeed, we have found immune complexes containing hepatitis-B antigen and IgG in the sera of a PAN patient studied.[2]

The nature of the serum blocking factors present in the SLE patients was further elucidated by immunoadsorption and fractionation studies. Removal of IgG but not IgM resulted in a marked decrease in ability of SLE sera to inhibit normal ADCC, suggesting that the blocking factors contain IgG but not IgM. We further demonstrated that both 7S and heavier gradient fractions containing IgG inhibited normal ADCC. Soluble immune complexes containing IgG can inhibit ADCC (21) by saturation of the K cell Fc receptors, specific for activated Fc of IgG. Inhibition of ADCC by the heavier fractions from SLE sera is probably due to immune complexes or aggregated IgG. A second mechanism for inhibition might involve IgG antibodies specific for cell surface antigens. These could bind to cells and inhibit ADCC acting as insoluble cellular immune complexes. Such a mechanism for inhibiting ADCC has been demonstrated in mice (22). The inhibition of normal lymphocyte ADCC activity by 7S fractions of SLE sera was probably due to the presence of antilymphocyte antibodies of the IgG class. Such antibodies are known to exist in SLE (23) and are probably responsible for inhibition of the mixed lymphocyte reaction. A third mechanism might involve antibodies specific for K cells which could sterically interfere with functional sites on these effector cells.

The reduction of ADCC activity in active SLE patients does not appear to be due to a corticosteroid effect, since we found no correlation between the dose of prednisone and ADCC activity. Furthermore, inactive SLE patients receiving up to 30 mg/day prednisone had normal ADCC activity.

Inhibition of ADCC by sera containing immune complexes may be a useful assay in patients with immune complex diseases. This was shown by examining SLE sera in periods of greater or lesser disease activity. In all cases, sera from patients in the more active phase of their disease showed greater inhibition of ADCC. Thus the inhibition of ADCC by patient's sera may be a useful guide to activity in immune complex related diseases.

REFERENCES

1. Perlmann, P., H. Perlmann, and H. Wigzell. 1972. Lymphocyte mediated cytotoxicity *in vitro*. Induction and inhibition by humoral antibody and nature of effector cells. *Transplant. Rev.* 13: 91–114.
2. MacLennan, I. C. M. 1972. Antibody in the induction and inhibition of lymphocyte cytotoxicity. *Transplant. Rev.* 13: 67–90.
3. O'Toole, C., V. Stejskal, P. Perlmann, and M. Karlsson. 1974. Lymphoid cells mediating tumor-specific cytotoxicity to carcinoma of the urinary bladder. Separation of the effector population using a surface marker. *J. Exp. Med.* 139: 457–466.
4. Hersey, P., P. Cullen, and I. C. M. MacLennan. 1973. Lymphocyte-dependent cytotoxic antibody activity against transplantation antigens. *Transplantation (Baltimore).* 16: 9–16.
5. Calder, E. A., W. J. Penhale, D. McLeman, E. W. Barnes, and W. J. Irvine. 1973. Lymphocyte-dependent antibody-mediated cytotoxicity in Hashimoto Thyroiditis. *Clin. Exp. Immunol.* 14: 153–158.
6. Wasserman, J., L.-V. von Stedingk, P. Perlmann, and J. Jonsson. 1974. Antibody-induced *in vitro* lymphocyte cytotoxicity in Hashimoto's Thyroiditis. *Int. Arch. Allergy Appl. Immunol.* 47: 473–482.
7. Kohler, H., D. R. Kaplan, and D. S. Strayer. 1974. Clonal depletion in neonatal tolerance. *Science (Wash. D. C.).* 186: 643–644.
8. Gale, R. P., and J. Zighelboim. 1975. Polymorphonuclear leukocytes in antibody-dependent cellular cytotoxicity. *J. Immunol.* 114: 1047–1051.
9. Winchester, R. J., J. B. Winfield, F. Siegal, P. Wernet, and Z. Bentwich, H. Kunkel. 1974. Analyses of lymphocytes from patients with rheumatoid arthritis and systemic lupus erythematosus. Occurrence of interfering cold-reactive antilymphocyte antibodies. *J. Clin. Invest.* 54: 1082–1092.
10. J. Schneider, W. Chin, G. J. Friou, S. M. Cooper, B. Harding, R. L. Hill, and F. P. Quismorio. 1975. Reduced antibody-dependent cell-mediated cytotoxicity in systemic lupus erythematosus. *Clin. Exp. Immunol.* 20: 187–192.
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. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest.* 21: Suppl. 97: 77–89.

13. Larsson, A., P. Perlmann, and J. B. Natvig. 1973. Cytotoxicity of human lymphocytes induced by rabbit antibodies to chicken erythrocytes. *Immunology.* 25: 675–686.
14. Kedar, E., M. O. De Landazuri, and J. L. Fahey. 1974. Enzymatic enhancement of cell-mediated cytotoxicity and antibody-dependent cell cytotoxicity. *J. Immunol.* 112: 26–36.
15. Talal, N., and R. Pillarisetty. 1975. IgM and IgG antibodies to DNA, RNA, and DNA:RNA in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* 4: 24–31.
16. Ashman, R. F. 1975. Enzymatic modification of lymphocyte receptors for antigen. III. Loss of antigen-responsiveness with recovery after adoptive transfer. *Scand. J. Immunol.* 4: 337–342.
17. Wisloff, F., S. S. Frøland, and J. B. Natvig. 1975. Deficient lymphoid cell-mediated, PHA-induced cytotoxicity in rheumatoid arthritis patients. *Scand. J. Immunol.* 4: 303–307.
18. Wernet, P., R. Thoburn, A. Moore, and H. Kunkel. 1974. Proceedings of the Eighth Leukocyte Culture Conference. Blockage of responder and stimulator lymphocyte surface structures and the shedding phenomenon mediated by human antilymphocytic sera in tissue culture. Academic Press Inc., New York. 543–552.
19. Messner, R. P., F. D. Lindström, 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.
20. Gocke, D. J., K. Hsu, C. Morgan, S. Bombardieri, M. Lockshin, and C. L. Christian. 1970. Association between polyarteritis and Australia antigen. *Lancet.* 2: 1149–1153.
21. MacLennan, I. C. M. 1972. Competition for receptors for immunoglobulin on cytotoxic lymphocytes. *Clin. Exp. Immunol.* 10: 275–283.
22. Halloran, P., V. Schirrmacher, H. Festenstein. 1974. A new sensitive assay for antibody against cell surface antigens based on inhibition of cell-dependent antibody-mediated cytotoxicity. I. Specificity and sensitivity. *J. Exp. Med.* 140: 1348–1363.
23. Williams, R. C., Jr., J. D. Emmons, and E. J. Yunis. 1971. Studies of human sera with cytotoxic activity. *J. Clin. Invest.* 50: 1514–1524.