Clin. exp. Immunol. (1973) **14,** 193–202.

DEPRESSION OF CELL-MEDIATED IMMUNITY IN OLD AGE AND THE IMMUNOPATHIC DISEASES, LUPUS ERYTHEMATOSUS, CHRONIC HEPATITIS AND RHEUMATOID ARTHRITIS

B. H. TOH, I. C. ROBERTS-THOMSON, J. D. MATHEWS, SENGA WHITTINGHAM AND I. R. MACKAY

Clinical Research Unit of The Walter and Eliza Hall Institute of Medical Research; and The Royal Melbourne Hospital, Victoria 3050, *Australia*

(*Received* 8 *November* 1972)

SUMMARY

Cell-mediated immunity was assessed in man by summation of positive delayed hypersensitivity (DHS) responses to five test antigens, Candida, mumps, trichophyton, tuberculin and streptokinase-streptodornase (Varidase). The study included forty-four controls comprising healthy persons and hospital inpatients with minor illnesses, forty-five aged persons, twenty-four patients with active chronic hepatitis (ACH), nine with systemic lupus erythematosus (SLE) and fourteen patients with rheumatoid arthritis. For controls and the group with ACH, cell-mediated immunity was assessed also by transformation of blood lymphocytes with phytohaemagglutinin (PHA). The incidence of positive DHS responses to all antigens fell with advancing age, and responsiveness was significantly lower in all three groups of immunopathic disease, particularly ACH. Prednisolone was not associated with significantly depressed DHS reactions, but combined prednisolone and azathioprine were. Weak DHS responses were associated significantly with low titres of natural antibody to flagellin and with lower humoral immune responses to flagellin. Lymphocyte transformation in response to PHA in ACH was significantly less than in healthy controls. Thus certain autoimmune diseases show an immunological imbalance in the form of depressed cell-mediated immunity to extrinsic antigens and 'hyperactive' humoral immune responses to 'self' antigens and selected extrinsic antigens.

INTRODUCTION

Cell-mediated immunity (CMI), as assessed conventionally in man by the delayed cutaneous

This is Publication No. 1834 from The Walter and Eliza Hall Institute.

Correspondence: Dr I. R. Mackay, Clinical Research Unit, The Walter and Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia.

hypersensitivity (DHS) reaction after intradermal challenge, suffers the disadvantage that the usual antigens used are those which test established immunity (secondary responsiveness) rather than induction of delayed sensitivity (primary responsiveness). Of the other methods for measuring CMI, stimulation of blood lymphocytes by phytohaemagglutinin (PHA) and release of leucocyte migration inhibition factor(s) (MIF) have not been developed as standard tests of CMI; other procedures include induction of responsiveness to haemocyanin or dinitrochlorobenzene (DNCB), but the latter may cause severe cutaneous reactions. We have assessed expression of CMI by measuring cutaneous responses to a standard group of five reagents known to evoke a DHS reaction; the subjects were healthy persons, hospital inpatients, aged persons, and persons with autoimmune diseases. For one subgroup, the results from skin tests were correlated with those obtained by lymphocyte transformation with PHA.

MATERIALS AND METHODS

Subjects studied

Controls. Controls for the DHS studies comprised forty-four subjects, eighteen females and twenty-six males, aged from 19 to 75 yr, and included seventeen healthy persons, and twenty-seven non-debilitated inpatients with relatively minor illnesses, without cancer or disease of the immune system, not receiving corticosteroids or immunosuppressive drugs, and not having a serum urea level greater than 60 mg/100 ml. Controls for studies on lymphocyte transformation comprised twenty-three healthy persons, ten females and thirteen males, aged 18–44 yr.

Advanced age. These forty-five subjects, twenty-eight females and seventeen males, over 80 yr of age, were selected at random from an institution for the aged; they were not debilitated nor chronically ill.

Active chronic hepatitis (ACH). These twenty-four patients, eighteen females and six males, were aged 18–75 yr and fulfilled the diagnostic criteria described by Mackay (1968). At the time of testing, four patients were receiving prednisolone, 5–10 mg, and azathioprine, 50–100 mg, daily, one was receiving cyclophosphamide, 100 mg daily, seven were receiving prednisolone, 5–20 mg daily, and twelve were receiving neither drug; all were stable in the remission phase. The indices of liver function used were serum glutamic oxaloacetic transaminase (GOT) determined by the Babson method, retention of a standard dose (5 mg/kg) of brom sulphalein (BSP) after 45 min, and level of serum albumin measured by the Biuret procedure.

Systemic lupus erythematosus (SLE). These nine patients, all female, were aged 20–66 yr and fulfilled the diagnostic criteria described by Dubois (1966). Four were receiving prednisolone, 7–10 mg, and azathioprine, 5–150 mg, daily, one was receiving azathioprine 150 mg daily, three were receiving prednisolone, 7·5–15 mg daily, and one was receiving neither drug; all were in remission.

Rheumatoid arthritis (RA). These fourteen patients, five females and nine males, were aged 27–72 yr and fulfilled the American Rheumatism Association criteria for definite or classical rheumatoid arthritis (Ropes *et al.*, 1959). One was receiving prednisolone, 14 mg, with azathioprine, 200 mg, daily, six were receiving prednisolone, 3–15 mg daily, and seven were receiving neither drug.

Immunological procedures

Delayed hypersensitivity skin tests. The five antigens used were Candida (Bencard), mumps skin testing antigen (Eli Lilly), trichophyton (Bencard), tuberculin 1:1000 strength (Commonwealth Serum Laboratories, Melbourne), and streptokinase 10 U—streptodornase 2·5 U (Varidase, Lederle). Each antigen, 0·1 ml, was injected intradermally, using a 1-ml syringe with a 26-gauge needle, in alphabetical order at 3–4-cm intervals down the forearm starting 4 cm from the distal elbow crease. Reactions were read at 48 hr after injection; the reaction was read as positive when the mean of the maximum and minimum diameters of the area of induration was greater than 0·6 cm.

Humoral response to flagellin. The procedure was as described by Rowley & Mackay (1969). Comparisons were made between titres of natural antibody and peak immune antibody to flagellin, and responses to the five skin test antigens.

Transformation of lymphocytes with PHA. Lymphocytes were separated from peripheral blood by centrifugation in an isopaque-ficoll gradient (Boyum, 1968). Thirty millilitres of blood were collected in Falcon tubes to which phenol-free heparin (5 units/ml) had been added. Four millilitres of blood were mixed with 4 ml of saline. Two millilitres of isopaque-ficoll were layered beneath this mixture and centrifuged at 400 *g* for 10 min at room temperature. The buffy coat was removed and the cells washed once in saline at 400 *g* for 5 min at 4°C. The cell pellet was resuspended in Eagle's medium with 10% foetal calf serum. The volumes were adjusted to give 1×10^6 lymphocytes/ml and dispersed in sterile Falcon tubes in duplicate. Duplicate cultures were set up with PHA (Burroughs Wellcome) using 0·5 μg, 20 μg and 100 μg. The lymphocytes were cultured at 37°C in 5% CO_2/95% air for 72 hr. One microcurie of tritiated thymidine per culture was added 6 hr before termination of culture. Excess thymidine was removed by washing the lymphocytes in 10 ml of Dulbecco's salt solution and centrifuging at 400 *g* for 5 min. The RNA was hydrolysed with 0·5 ml 2 M KOH at room temperature for 30 min. DNA was extracted with trichloracetic acid (TCA), 2 ml 40% TCA at 4°C for 2 hr followed by centrifugation at 4500 rev/min at 4°C for 25 min; the pellet was resuspended in 0·5 ml 10% TCA. The extract was dried at 55°C for 45 min. 0·5 ml soluene was added and the mixture placed in a warm oven for 1 hr. The solubilized DNA was transferred into 10 ml scintillation fluid in precounted bottles, and counted in a Packard liquid scintillation spectrometer. Results were expressed as disintegrations/minute of tritiated thymidine incorporated into DNA. Dose response curves to PHA were derived as described by Fitzgerald (1971).

Statistical tests

The χ^2 test was used to compare numbers of positive results between groups tested with the five skin test antigens. Student's *t*-test was used to compare (i) mean values for groups of patients and controls of disintegrations per min of thymidine after transformation of lymphocytes by PHA at various doses, and (ii) mean titres of antibody to flagellin in patients grouped according to their DHS reactions. The results of PHA transformation tests and the titres of antibody to flagellin were transformed logarithmically beforehand.

RESULTS

DHS responses to five antigens

Controls. All except one of the forty-four controls responded to three or more of the

five antigens, and one responded to one antigen only (Fig. 1). Within this group, the responses of healthy controls and hospital controls were similar, as were those of males and females. Most of the controls (>70%) responded to each test antigen except for trichophyton to which only fourteen of twenty-six males and one of eighteen females responded.

Advanced age. Positive DHS reactions to the five antigens in the forty-five subjects over 80 yr of age were markedly decreased in that sixteen failed to react to any, thirteen reacted only to one, eleven to two, and only two to three or more antigens (Fig. 1); this decrease was not related to any one particular antigen (Table 1). Mean values for the number of positive skin test results per decade, considering controls and aged subjects together, showed a gradual fall for each decade between 20–30 and 90–100 yr (Fig. 2).

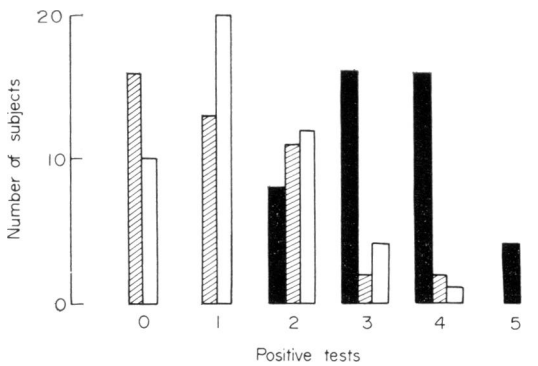

FIG. 1. DHS reactions to five antigens in (solid columns) control subjects, (diagonal hatched) aged subjects and patients with (open hatched columns) active chronic hepatitis (ACH), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA); reactivity in aged subjects and patients is markedly decreased.

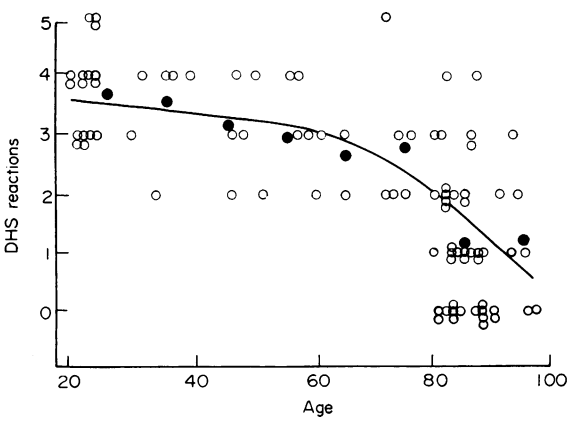

FIG. 2. Mean number of positive DHS reactions per decade in groups of control and aged subjects, showing progressive fall with age in the prevalence of positive cutaneous reactions to the five antigens (Candida, mumps, trichophyton, tuberculin and Varidase). (○) Independent values; (●) mean values per decade.

TABLE 1. Incidence and percentage of positive DHS reactions to five antigens in groups studied

Antigen	Groups (No. of cases)				
	Controls (44)	Advanced age (45)	ACH* (24)	SLE* (9)	RA* (14)
Candida	32 (73%)	15 (33%)	5 (20%)	4 (44%)	8 (57%)
Mumps	35 (80%)	15 (33%)	5 (20%)	3 (33%)	6 (43%)
Trichophyton	14 (32%)	2 (3%)	1 (4%)	0	1 (7%)
Tuberculin	33 (75%)	12 (27%)	7 (30%)	0	7 (50%)
Varidase	34 (77%)	9 (20%)	9 (38%)	3 (33%)	3 (21%)
All antigens	148 (70%)	53 (24%)	27 (20%) †Exp. 75‡	10 (22%) †Exp. 33‡	25 (42%) †Exp. 42§

* ACH = active chronic hepatitis, SLE = systemic lupus erythematosus, RA = rheumatoid arthritis.

† Exp. = expected number of positive skin tests to five antigens for disease group if there were no association between disease and skin test reactivity; this calculation (corrected for age and sex) was based on the results of tests in controls and subjects of advanced age.

‡ $P<0{\cdot}001$; § $P<0{\cdot}01$.

Immunopathic diseases. Considering all five antigens, the twenty-four patients with ACH, nine with SLE and fourteen with RA, showed fewer responses than did controls, particularly so for ACH, less so for SLE and least so for RA. Ten of the forty-seven patients with these diseases were unresponsive to all five antigens (Figs 1 and 3), in contrast to none of the forty-four controls. Considering single antigens, the responses of patients with ACH were poor to all five, responses of patients with SLE were particularly poor to tuberculin and mumps, whereas responses of patients with rheumatoid arthritis were particularly poor to the streptococcal antigen Varidase.

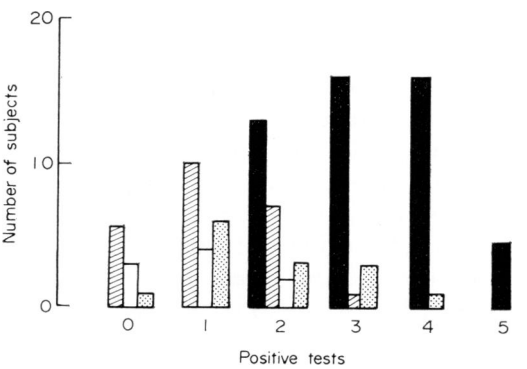

FIG. 3. DHS reactions of controls and patients with immunopathic diseases (scored as in Fig. 1) showing greatest depression of responsiveness in patients with ACH and least in RA (solid columns) controls; (diagonal hatched columns) ACH; (open columns) SLE; (stippled columns) RA.

Degree of skin response to antigens. Considering all positive responses for the groups of controls, advanced age and patients, there were no differences between groups in the degree of cutaneous response, as assessed by the diameters of induration.

Drug therapy and cutaneous responses. Considering pooled results for all three patient groups, there were no differences in numbers of positive reactions according to whether or not patients were receiving prednisolone. However there were significantly fewer reactions in patients receiving prednisolone and azathioprine than in those receiving neither of these drugs. This effect did not account for the overall difference between controls and patients in that responses for patients not receiving these drugs were still significantly less than those for controls (Table 2).

TABLE 2. Number of positive DHS responses to five antigens and treatment received in patients with SLE, ACH and RA

Groups*	Incidence and percent (in parentheses) of positive DHS tests
Group 1 (18)	29/90 (32)
Group 2 (16)	17/80 (21) (ns)
Group 3 (9)	6/45 (13)†

* Group 1, no prednisolone or azathioprine; group 2, prednisoline 3–20 mg/day; group 3, prednisolone 5–14 mg/day and azathioprine 50–200 mg/day. Number of cases in parentheses.

† $P<0{\cdot}05$, compared with group 1.

ns = not significant.

Transformation of lymphocytes with PHA

The dose response curve for the transformed lymphocytes of the controls and patients with ACH is shown in Fig. 4. Of the patients with ACH, there was normal transformation of lymphocytes in two, depressed transformation in the unstimulated cultures in three, depressed transformation with five or 20 μg of PHA in three, and depressed transformation with 5, 20 and 100 μg of PHA in four. Only two of the seven patients showing a depressed lymphocyte response to PHA were receiving azathioprine. These differences between patients with ACH and controls were statistically significant ($P = <0{\cdot}001$) for unstimulated cultures, and maximally stimulated cultures ($P<0{\cdot}005$) (Fig. 4). These results of *in vitro* tests are in accord with the low DHS responses of patients with ACH, but there was no individual correlation between depression of PHA transformation and cutaneous DHS.

Correlation of humoral response to flagellin and DHS response to five antigens

For patients with immunopathic diseases there was a trend for low DHS responsiveness to be associated with low titres of antibody to flagellin, and this was significant for natural antibody (pre-immunization), as shown in Table 3.

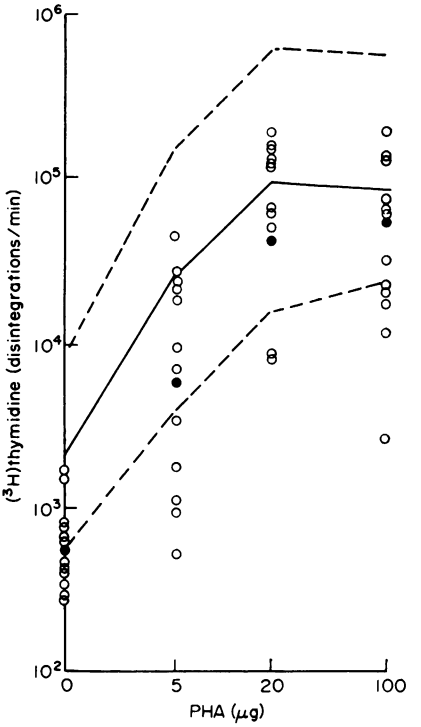

FIG. 4. Dose-response curves for lymphocyte transformation by PHA in controls (mean ——, ±2 SD - - -) and patients with ACH for whom depression of transformation was statistically significant for unstimulated cultures ($P<0{\cdot}001$) and maximally stimulated (100 μg PHA) cultures ($P<0{\cdot}005$). (○) Independent values; (●) mean values.

TABLE 3. Comparison of 'low' and 'high' DHS reactivity with humoral immune responses to flagellin

DHS reactivity*	Response to flagellin				
	Pre-immunization (natural antibody)	Primary		Secondary	
		2 Weeks	10 Weeks	2 Weeks	10 Weeks
'Low' (15)†	31 (27–42)	1032 (252–3980)	242 (53–1200)	3054 (912–10470)	1607 (324–4070)
'High' (6)	230‡ (34–1560)	2754 (1660–4570)	659 (66–6590)	7943 (1700–37150)	1750 (537–13490)

* Low = 0–1 positive reactions and high = ⩾2 positive reactions; number in group (in parentheses).

† Eleven of these in secondary responses.

‡ $P<0{\cdot}01$.

Indices of disease and skin test response in ACH

There were no correlations between functional indices of liver disease and DHS responses in ACH. For example, the mean values for BSP retention for six patients responding to none of the five antigens was 23%, for ten patients responding to one of the five antigens was 31%, and for seven patients responding to two or more of the five antigens was 24%.

DISCUSSION

The DHS responses for the controls in this study are generally similar to those reported for an Australian population by Forbes (1971) in that all except one of our controls reacted to more than two antigens, and the incidence of positive reactions to Candida, mumps, trichophyton and Varidase was 75, 80, 32 and 77%; the incidence of tuberculin reactors of 75% was considerably higher than the 24% reported by Forbes (1971). The low incidence of responses to trichophyton in females may reflect a low contact rate with this fungus in females. The proportion of positive skin tests declined with age, particularly so in the seventh and eighth decades. Lack of cutaneous DHS reactivity in the aged to tuberculin is known; Bellomo (1952) tested 2000 persons with PPD 1:1000 and found proportionately fewer reactors in the sixth decade of life, and Harris (1967) tested 171 subjects with PPD 0·001 mg and found a significant decrease in reactivity over the range of 70–90 yr. The other antigens used in this study do not appear to have been assessed in aged subjects.

Of the immunopathic diseases tested, depression of DHS reactions was most marked with active chronic hepatitis, moderate with SLE and least with rheumatoid arthritis; this could not be accounted for by differences in age, sex or treatment among these three groups. The marked depression of DHS reactivity in ACH could not be related to decreased liver function in that depression of reactivity in our study did not correlate with biochemical indices of liver function, and Straus *et al.* (1971) using an even greater range of test antigens than we used, found normal cutaneous DHS reactions in fifty patients with alcoholic cirrhosis.

Previous studies on DHS reactions in immunopathic diseases have pointed towards decreased reactivity, although results have been somewhat inconsistent. Block *et al.* (1968) studied twenty patients with SLE, twenty-three with rheumatoid arthritis and 112 hospital controls, and found a significantly decreased reactivity to tuberculin in SLE, but otherwise no differences between patients and controls in the frequency of positive reactions to PPD, histoplasmin, trichophyton, Candida or streptokinase. Horwitz (1972) observed marked depression of DHS responses to Candida, Varidase and trichophyton in fourteen patients with SLE, most of whom were tested soon after diagnosis and before prednisolone was given. Hayes, Ward & Jennings (1970) studied sixty-two patients with rheumatoid arthritis and eight-four controls and found the incidence of DHS reactions to tuberculin, mumps and brucella to be significantly depressed in rheumatoid arthritis; this was attributed to the greater functional incapacity of the patients. Whaley *et al.* (1971) used DNCB to sensitize patients with rheumatoid arthritis and found that skin reactivity was decreased relative to control subjects. Bitter (1971) using a wide range of antigens found depression of DHS in patients with SLE and rheumatoid arthritis in line with the present findings. In patients with ACH, the depression of lymphocyte transformation *in vitro* was a further indication of impaired function of lymphocytes involved in CMI, and corroborated the findings derived from skin tests for DHS. Martini *et al.* (1970) likewise reported depressed synthesis

of both RNA and DNA in lymphocytes of patients with chronic hepatitis. However, it is likely that skin tests and transformation response to PHA measure different aspects of cellular immune function; Daguillard (1972) cited a few instances of patients with thymic deficiency syndromes having normal responses to PHA despite clearly depressed DHS responses. Also, Horwitz (1972) found a marked depression of DHS responsiveness in SLE (*vide supra*), but the same patients showed similar lymphocyte responses to PHA as did the control patients with tuberculosis.

Impaired expression of cell-mediated immunity in immunopathic disease could represent an immunosuppressive effect of the disease itself or its treatment (but from our results an effect of treatment *per se* seemed unlikely), or alternatively a pre-existing deficiency in the cellular immune (T cell) system which could predispose to such diseases (Burnet, 1969; Allison, Denman & Barnes, 1971). It was claimed by Cooper, Peterson & Good (1970) that autoimmune disorders occurred with greater than expected frequency in children and adults with frank immunological deficiency, and that neonatal thymectomy in animals predisposed to various autoimmune states. Ziff (1971), Allison *et al.* (1971) and Fudenberg (1972) suggested, each in a somewhat different form, that human autoimmune diseases could result from genetically determined thymic-dependent immunological deficiency states facilitating a pathogenic effect of an environmental agent, presumably a virus. Findings of raised antibody titres to viral antigens, e.g. to measles virus, in SLE (Lucas *et al.*, 1972) are consistent with either a causal effect of such viruses, or an increased predisposition to viral infection in established autoimmune disease.

We do not see the possibility on present evidence of unravelling the complex interrelations between autoimmunity and immunodeficiency. No more can be said than that autoimmune disorders are associated, either causally or consequentially, with an immunological imbalance in the form of depressed CMI to extrinsic antigens and augmented (non-controlled) humoral immune responses to 'self' antigens and certain viral antigens.

ACKNOWLEDGMENTS

We are indebted to Eli Lilly for their generous gift of mumps testing antigen. We thank Mrs Dorothy Goriup and Miss Marjorie Crawford for expert technical assistance, and Miss Ida Langford, S.R.N., for help with patients. I.R.M., S.W. and J.D.M. are in receipt of a grant from the National Health and Medical Research Council of Australia.

REFERENCES

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971) Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet*, **ii**, 135.

BELLOMO, J. (1952) Incidence of tuberculin reactions in a series of 2,000 patients seen in a private hospital of internal medicine. *Dis. Chest*, **21**, 179.

BITTER, T. (1971) A clinical investigation of the pathogenesis of autoimmunity. *Rheumatoid Arthritis* (Ed. by W. Muller, H. G. Harwerth and K. Fehr), p. 305. Academic Press, London and New York.

BLOCK, S.R., GIBBS, C.B., STEVENS, M.B. & SHULMAN, L.E. (1968) Delayed hypersensitivity in systemic lupus erythematosus. *Ann. rheum. Dis.* **27**, 311.

BOYUM, A. (1968) Separation of leucocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21**, *Suppl.* 97.

BURNET, F.M. (1969) *Cellular Immunology*. Melbourne University Press, Melbourne.

COOPER, M.D., PETERSON, R.D.A. & GOOD, R.A. (1970) Development and physiology of the lymphoid system. *The Immunochemistry and Biochemistry of Connective Tissue and its Disease States. Rheumatology: An Annual Review* (Ed. by J. Rotstein), p. 86. S. Karger, Basel.

DAGUILLARD, F. (1972) Immunologic significance of *in vitro* lymphocyte responses. *Med. clin. N. Amer.* **56**, 293.

DUBOIS, E.L. (1966) Differential diagnosis, criteria for diagnosis and classification of systemic lupus erythematosus. *Lupus Erythematosus* (Ed. by E. L. Dubois), McGraw-Hill, New York.

FITZGERALD, M.G. (1971) The establishment of a normal human population dose-response curve for lymphocytes cultured with PHA. *Clin. exp. Immunol.* **8**, 421.

FORBES, I.J. (1971) Measurement of immunological function in clinical medicine. *Aust. N. Z. J. Med.* **1**, 160.

FUDENBERG, H.H. (1972) Are autoimmune diseases immunologic deficiency states? *Immunobiology: Current Knowledge of Basic Concepts in Immunobiology and their Clinical Applications* (Ed. by R. A. Good and D. W. Fisher), p. 175. Sinaver Associates Inc., Stamford, Connecticut.

HARRIS, C. (1967) Evaluation of tuberculin testing in the elderly. *J. Amer. Geriat. Soc.* **15**, 828.

HAYES, J.R., WARD, D.J. & JENNINGS, J.F. (1970) Studies on cell-mediated hypersensitivity responses in rheumatoid arthritis. *Impaired Cell-Mediated Hypersensitivity in Man* (Ed. by J. F. Jennings and D. J. Ward), p. 37. W. F. Crane Ltd, Oswestry.

HORWITZ, D.A. (1972) Impaired delayed hypersensitivity in systemic lupus erythematosus. *Arthr. and Rheum.* **15**, 353.

LUCAS, C.J., BROUWER, R., FELTKAMP, T.E.W., TEN VEEN, J.H. & VAN LOGHEM, J.J. (1972) Measles antibodies in sera from patients with autoimmune diseases. *Lancet*, **i**, 115.

MACKAY, I.R. (1968) Lupoid hepatitis and primary biliary cirrhosis: autoimmune diseases of the liver? *Bull. rheum. Dis.* **18**, 487.

MARTINI, G.A., RÖSSLER, R., HAVEMANN, K. & DÖLLE, W. (1970) Inhibited lymphocytic response to phytohemagglutinin (PHA) in acute infectious hepatitis and in chronic aggressive hepatitis; indirect evidence for virus persistence in chronic aggressive hepatitis? *Scand. J. Gastroent. Suppl.* **7**, 39.

ROPES, M.W., BENNETT, G.A., COBB, S., JACOX, R. & JESSAR, R.A. (1959) Revision of diagnostic criteria for rheumatoid arthritis. *Ann. rheum. Dis.* **18**, 49.

ROWLEY, M.J. & MACKAY, I.R. (1969) Measurement of antibody producing capacity in man. I. The normal response to flagellin from *Salmonella adelaide*. *Clin. exp. Immunol.* **5**, 407.

STRAUS, B., BERENYI, M.R., HUANG, J.-M. & STRAUSS, E. (1971) Delayed hypersensitivity in alcoholic cirrhosis. *Amer. J. dig. Dis.* **16**, 509.

WHALEY, K., GLEN, A.C.A., MACSWEEN, R.N.M., DEODHAR, S., DICK, W.C., NUKI, G., WILLIAMSON, J. & BUCHANAN, W.W. (1971) Immunological responses in Sjøgren's syndrome and rheumatoid arthritis. *Clin. exp. Immunol.* **9**, 721.

ZIFF, M. (1971) Viruses and the connective tissue diseases. *Ann. intern. Med.* **75**, 951.