Clin. exp. Immunol. (1974) **17**, 427–436.

THE OCCURRENCE OF AUTOANTIBODIES IN INFECTIOUS MONONUCLEOSIS

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(*Received* 14 *January* 1974)

SUMMARY

Autoantibodies were looked for by immunofluorescence (IFL) in seventy-seven cases of infectious mononucleosis (IM) at the onset of symptoms and on recovery, to determine the time of appearance, duration and range of these responses, and to correlate them with serum immunoglobulin and EB virus antibody titres. Antibodies to lymphocyte membrane demonstrated by IFL, now identified with lymphocytotoxins, were present in 46% of patients in the acute stage, persisting for less than 7 weeks. Antibodies to smooth muscle (SMA) or to contractile fibres in other tissue cells including human thyroid and rat hepatocytes, were present in over 70% of cases, some being entirely of IgM class. The highest titres occurred soon after onset and these antibodies also disappeared during convalescence. By contrast ANA, mitochondrial, microsomal and reticulin antibodies, also thyroid and gastric organ-specific reactivity were seen only occasionally owing to the young age group of the patients. In individual cases there was no correlation between the appearance of lymphocyte antibodies and SMA, or between these and the EB virus antibody titres.

The autoantibodies produced in this disease are highly selected. It is suggested that clones of B cells are stimulated to make these antibodies by virtue of being infected with EB virus, and that the T-cell clones in the circulation are more likely expanded in order to terminate the infection.

INTRODUCTION

Autoantibodies, including antinuclear (ANA) (Holborow *et al.*, 1963; Carter, 1966), anti-IgG (RF) (Holborow *et al.*, 1963) anti-i (Wollheim & Williams, 1966), smooth muscle (Holborow, Hemsted & Mead, 1973) and anti-lymphocyte activity specific for subpopulations of autologous thymocytes and lymphocytes (Thomas, 1972), as well as cryo-

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proteins (Kaplan, 1968), have been observed in patients with infectious mononucleosis. In addition, high levels of immunoglobulins are present during the acute phase of the disease (Wollheim & Williams, 1966), and have been observed in a proportion of cases tested as long as 2 years after recovery (Sutton *et al.*, 1973).

In this paper, a comparison has been made between the incidence of anti-lymphocyte and tissue antibodies and levels of immunoglobulins, heterophile and anti-EB virus reactivity at onset of disease and after recovery.

MATERIALS AND METHODS

Patients and controls

The criteria for diagnosing infectious mononucleosis (IM) were clinical, together with a positive Paul–Bunnell or equivalent test, and the presence of atypical mononuclear cells in the peripheral blood. Five patients did not have heterophile antibodies but were included as they had all the features of infectious mononucleosis. One control group consisted of patients admitted to an infectious diseases hospital, and healthy controls were medical students and nurses at the beginning of their training.

Serological methods

Immunoglobulins. These were estimated by single immunodiffusion, slightly modified, from Mancini, Carbonara & Heremans (1965). Results were expressed as a percentage of the Medical Research Council standard for the three main immunoglobulin (Ig) classes.

Anti-lymphocyte antibodies. Antibodies reacting with a lymphocyte surface antigen were detected by indirect immunofluorescence (IFL) using a specific anti-human IgM–FITC conjugate, at 3°C with viable suspensions of human tonsillar lymphocytes, as described by Thomas (1972). These antibodies, when present, have reacted with all tonsillar suspensions (at least fifty separate operative specimens) and the proportion of fluorescent lymphocytes in different preparations varied between 30 and 50%. The anti-IgM conjugate was adjusted to a dilution which gave minimum direct immunofluorescence (5–10%) and was presumed to be B cells. With each individual suspension a positive IM reference serum (H1-S) was included together with three normal sera to establish the number of lymphocytes reacting with IM sera and the proportion staining with conjugate only. As an extra precaution all weakly reactive IM sera were considered as negative.

Tissue antibodies. Autoantibodies including thyroid, gastric parietal cell, mitochondrial (M), liver–kidney microsomal (Rizzetto, Swana & Doniach, 1973), antinuclear (ANA) and the various reticulin antibodies (Seah *et al.*, 1973; Rizzetto & Doniach, 1973) as well as antibodies related to smooth muscle (SMA) proteins (Trenchev, Sneyd & Holborow, 1974) were all detected by immunofluorescence using 5 μm cryostat sections from a composite block of human thyroid and stomach, and rat liver and kidney. Sera were tested at an initial dilution of 1:10 with a polyvalent anti-human γ-globulin–FITC conjugate. Weakly reacting sera were regarded as negative.

Other tests. Rheumatoid antiglobulins (RF) were assayed by Latex FII slide test (Wellcome Reagents). Serum bilirubin, alkaline phosphatase and aspartate transaminase were estimated by standard methods in cases where liver involvement was suspected clinically. Antibodies to EB virus capsid antigen (VCA) and EB complement-fixing (CF) antibodies were detected by methods described previously (Sutton *et al.*, 1973).

Statistical analysis. Each batch of sera contained comparable numbers of patients and controls which had been randomized (Fisher & Yates, 1963) and these were tested in a double-blind manner. Statistical methods included Student's t-test, the χ^2 test with Yates' correction, Fisher's exact test and the Spearman rank correlation coefficient (Siegel, 1956).

RESULTS

Anti-lymphocyte antibodies

Ninety-seven sera from seventy patients with IM were tested for cold IgM antibodies reacting with subpopulations of thymocytes and blood lymphocytes. These antibodies were present in thirty-two out of seventy patients (46%) during the active phase of illness, but did not show any correlation with smooth muscle or other autoantibody titres. They declined rapidly upon clinical recovery and were never found later than 7 weeks, after the onset of symptoms (Fig. 1).

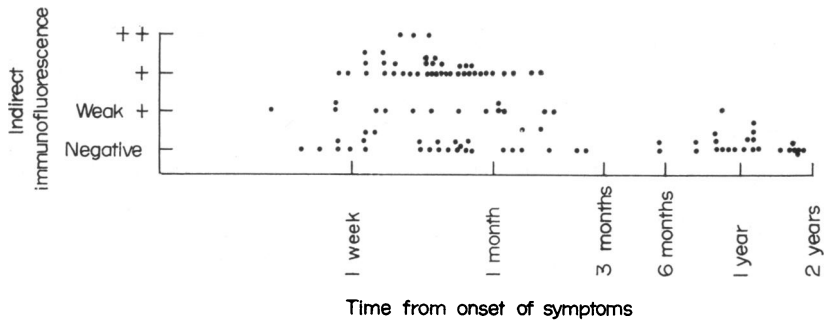

FIG. 1. Antibodies to lymphocyte membrane. Weak reactions are shown but were not included in calculations.

This time-consuming immunofluorescent assay could not be applied to the control groups included in this paper but in the past 3 years sixty-eight healthy controls from laboratory staff and selected hospital patients have been assayed and found to be negative.

Tissue antibodies

One hundred and five sera from seventy-seven IM patients, 103 sera from ninety-eight patients with other infections and sera from ninety healthy medical students and nurses were tested.

Smooth muscle fluorescence (SMA)

In patients with active IM, tested less than 30 days after onset of symptoms, thirty-eight out of seventy-seven individuals (49%) showed strong smooth muscle fluorescence with anti-γ; however, when thirty of the negative sera were retested with monospecific anti-IgM, fourteen gave positive results. The highest titres (80) were found early on (Fig. 2) and the antibodies disappeared on recovery, the longest persistence being 7 weeks. Reversal of positive fluorescence was demonstrated in eighteen out of thirty-five patients; three cases which were initially negative had traces of antibody 1 and 2 years after recovery, and thirteen patients were negative during both the illness and later.

In control patients, seven out of thirteen with infective hepatitis (54%) had smooth

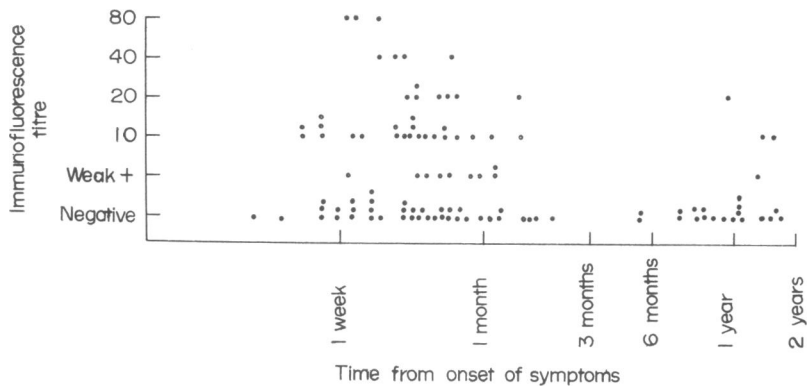

FIG. 2. Smooth muscle fluorescence (SMA).

muscle antibodies in titres up to 80 but overall the proportion of positive reactors was lower in the seventy-four control patients with other diagnoses, of whom six had smooth muscle fluorescence (8%). Fourteen of these patients were tested again in convalescence, and all were negative.

Eleven of ninety healthy medical students and nurses had smooth muscle antibodies in titres of 10–40 (12%), a proportion similar to that of control patients and much lower than that observed in infective mononucleosis or infective hepatitis.

The levels of serum immunoglobulin in patients with and without smooth muscle antibodies were analysed. The geometric mean values of IgM and IgG were higher in the groups with SMA than in those without, that of IgM reaching statistical significance in the control groups.

IFL patterns related to smooth muscle proteins

Apart from fluorescence of smooth muscle fibres, several IFL patterns observed on tissue sections have now been attributed to antibodies reacting with six separate smooth muscle proteins (Trenchev *et. al*, 1973). Some of these were looked for in the present series:

Polygonal. This SMA-related antibody gives a continuous line of staining around rat hepatocytes. It was found in eighteen patients with IM, tested from 6 to 570 days after the onset of symptoms, twelve of whom also had SMA in titres of 10–80. One patient possessed these antibodies at 20 and 163 days after the onset of illness. Of the control groups, hexagonal IFL was found in four out of thirteen cases of infective hepatitis and one with carcinoma of the pancreas but in none of the ninety healthy controls.

Bile canalicular fluorescence, i.e. a discontinuous double line of staining, around rat hepatocytes, was found with sera from two IM patients tested 6 and 17 days after onset, and in none of the control patients or healthy individuals.

Glomerular. Several appearances occur with sera from patients with chronic liver disease and high SMA titres. None of the IM cases or controls were positive.

Thyroid pericellular (Fig. 3). IFL was observed in thirty out of sixty-three patients with acute infectious mononucleosis (48%), in twelve out of eighty-eight control patients (14%), six of whom had infective hepatitis, and in five out of ninety healthy subjects (6%). There was some correlation between these antibodies and smooth muscle fluorescence (Fig. 4).

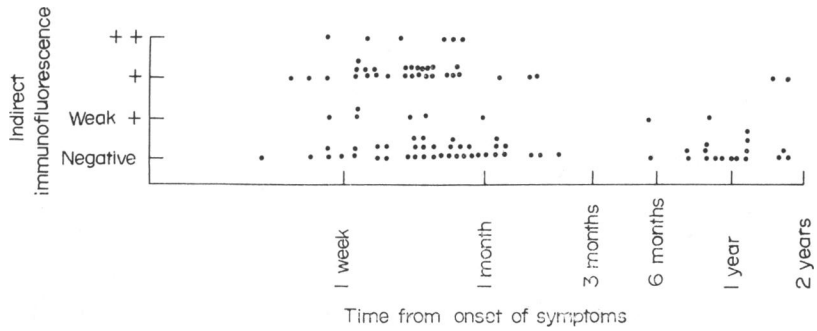

FIG. 3. Thyroid pericellular fluorescence.

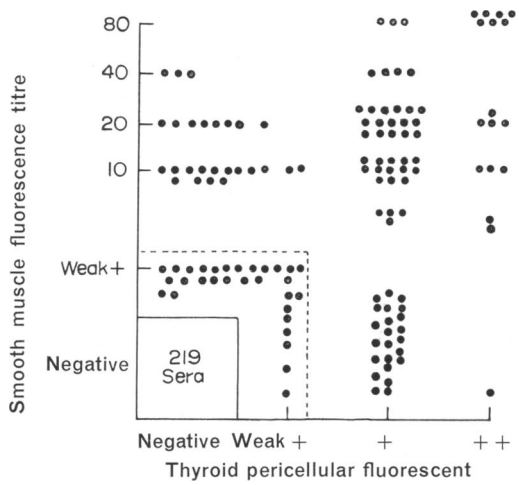

FIG. 4. Correlation between SMA and thyroid pericellular fluorescence.

Reticulin antibodies. Reticulin fluorescence of the main pattern (R_1) described in coeliac disease (Seah *et al.*, 1973) was found in six IM sera, in two patients with infective hepatitis, one with rubella, and in one healthy control. A second fibrillary pattern (named R_2 in Rizzetto & Doniach, 1973) characterized by fine sharp fibres around portal tracts and surrounding vessels in rat kidney and liver and, unlike R_1, showing no cross-reaction with human tissues, was seen in one IM patient, six patients with other infections and one healthy control. A third reticulin pattern characterized by staining of hepatic sinusoidal walls, Kuppffer cells and blood-derived adherent monocytes, named the R_s pattern, was found in two healthy subjects only. When all these patterns are considered together reticulin antibodies were found in 10% of IM cases and patients with other infections and 5% of healthy controls.

Other tissue antibodies. Non-organ-specific reactions such as ANA antiglobulins (RF), mitochondrial, and liver/kidney microsomal antibodies were rarely present in any of the three groups studied (Table 1) and do not appear to be stimulated during the active phase of IM. The same applies to organ-specific reactions to thyroglobulin & thyroid microsomes, which were found in 8% of the IM patients all in low titres, and gastric parietal cells

TABLE 1. Autoantibodies in patients with infective mononucleosis and in control groups

Antibodies to:	Subjects tested:		
	Infectious mononucleosis	Control patients	Healthy controls
Lymphocyte membrane (T-cell subpopulation)	32/70	0/68*	0/68*
Smooth muscle (SMA)	38/77	13/98†	12/90
SMA related patterns			
Thyroid pericellular	32/77	12/98	4/90
Hexagonal (hepatocytes)	15/77	5/98	0/90
'Bile canalicular'	2/77	0/98	0/90
Glomerular	0/77	0/98	0/90
Nuclei (ANA)	1/77‡	3/98	0/90
Mitochondria	0/77	0/98	1/90
Liver/kidney microsomes	0/77	0/98	0/90
Immunoglobulins (RF, Latex, FII)	1/76	2/92	4/91
Thyroglobulin (TRC)	5/69	3/97	4/80
Thyroid microsomes	1/76	2/98	1/90
Gastric parietal cells	3/77	4/98	1/90

* Not the same patients or controls.
† 7/13 with infectious hepatitis, 6/85 without hepatitis.
‡ Paul–Bunnell negative mononucleosis.

present in 4%. Of the thirty-five IM patients retested after recovery two had weak gastric PC fluorescence initially, which was not seen in the late specimen. No patients with thyroid antibodies were available for retesting.

Clinical features and their relation to tissue antibodies.

Analysis of the clinical features in sixty-six of the patients with infectious mononucleosis showed that the mean duration of illness was 24·3 days. The severity of illness was assessed in twenty-one patients (32%) as mild, in thirty-nine patients (59%) as moderate and in six patients (9%) as severe. There were no deaths but the disease was complicated in individual patients by myocarditis, respiratory obstruction, haemolytic anaemia and by splenic rupture. There was a significant correlation ($\chi^2 = 7{\cdot}15$; $P<0{\cdot}01$) between the administration of ampicillin and the development of a rash, but otherwise there was no discernible relation between the severity of illness and any other observation, in particular, the development of tissue antibodies. In twenty patients, eighteen of whom were considered to be severely or moderately ill, liver function tests were carried out. Four out of seven patients with serum bilirubin levels of 1·0 mg% or over possessed smooth muscle antibodies, as did five of eleven patients with serum bilirubin levels below 1·0 mg %; these figures do not differ significantly ($P = 0{\cdot}33$). There was no rank correlation between either serum alkaline phosphatase values and smooth muscle antibodies ($r_s = 0{\cdot}031$; $P<0{\cdot}7$) or between serum aspartate

transaminase levels and smooth muscle antibodies ($r_s = 0{\cdot}061$; $P<0{\cdot}9$), and there was no correlation between the sera with alkaline phosphatase (P–0·4; exact test) or aspartate transaminase levels ($P = 0{\cdot}3$; exact test) outside the normal range and the presence or absence of smooth muscle antibodies.

EB virus studies

Antibodies to EB virus capsid antigen and to EB virus-soluble complement-fixing antigen were estimated in all patients; either or both of these antibodies were detected in all twenty-two patients with infectious mononucleosis who were tested late in convalescence. Further details were given by Sutton *et al.* (1973). There was no correlation between the titres of EB virus antibodies and the tissue reactivities described in the present study.

DISCUSSION

The purpose of this study was to follow the appearance and duration of various autoantibody activities in infectious mononucleosis (Lancet, 1973) in relation to the rise in serum immunoglobulins and EB virus antibodies (Sutton, 1972).

The lymphocyte membrane-specific antibody first detected by immunofluorescence in IM sera (Thomas, 1972) has recently been shown to correlate closely with the lymphocytotoxins described by Mottironi & Terasaki (1970). Both activities are due to cold IgM antibodies, both are present in infectious mononucleosis, collagen disorders (Terasaki, Mottironi & Barnett, 1970) and cold agglutinin disease or cryoglobulinaemia. The membrane fluorescence and lymphocytotoxic tests have now been carried out concurrently in a series of patients (Thomas, 1973). A close correspondence was found between the two tests, regarding both the percentage of reactive cells in any given sample of tonsillar lymphocytes and the titre of the antibodies in the serum, suggesting that the two assays are measuring one and the same antibody activity. Furthermore, using immunoabsorbent columns which were able to separate B and T lymphocytes from each other, it has been confirmed that the lymphocyte membrane antibodies are specific for a subpopulation of T cells. In addition, some IM sera contain an anti-lymphocyte antibody which appears to be specific for B blasts (Thomas & Phillips, 1973) and is distinct from the antibody studied in the present paper. The lymphocyte membrane antibody was detected as early as 2 days after onset of symptoms and disappeared again 3–8 weeks later, being found with equal frequency in male and female patients. Its presence did not correlate with that of any other type of antibody in individual sera.

An increased incidence of smooth muscle fluorescence has been reported in IM by several authors. Holborow *et al.* (1973) found these antibodies in 81% of 126 patients tested within 1 month after onset of symptoms. Our lower incidence is due partly to elimination of weak positive reactions and to the lesser sensitivity of our polyvalent conjugate to IgM antibodies. In other respects such as the incidence in normal controls, range of titres, and rate of disappearance of the antibodies on recovery, our results agree entirely with these authors.

The highest SMA titres occurred in the first week of illness in IM, as previously observed in HA viral hepatitis (Adjukiewicz *et al.*, 1972) and in HB hepatitis (Holborow, 1972a). The same applies to hepatitis and mononucleosis due to cytomegalovirus infections. It appears that many different viruses can elicit the formation of SMA; these cannot be exclusively related to hepatocyte damage since they are found in patients with cancer with

no liver involvement (Whitehouse & Holborow, 1971). Many other tissue cells contain actomyosin-like contractile fibrils (Holborow, 1972b) and the work of Trenchev *et al.* (1974) suggests that human sera contain several closely related antibodies reacting with other smooth muscle proteins. Thyroid pericellular fluorescence appears to belong to this group, although the exact nature of the contractile protein antigen has not been established for these cells. This IFL pattern is sometimes seen with sera from chronic hepatitis patients having high titre SMA, but weaker reactions occur with SMA-negative specimens. The frequency of thyroid pericellular fluorescence was unusually high in active IM and became negative on recovery in parallel with SMA. Polygonal fluorescence of hepatocytes, and the unusual pattern simulating bile canaliculi were seen less frequently, and glomerular staining, which is also allied to SMA, was not seen at all in IM or the control groups. Perhaps viral infections reveal or alter the contractile proteins which lie immediately below the cell membrane, so that they become immunogenic. The role of the EB virus in this is not unexpected for infection with herpes simplex virus, itself closely related to the EB virus, is known to produce changes in the immunological specificity of infected cells and, also, by producing changes in the membrane structure, to alter the 'social' behaviour of the cells thus rendering them more 'malignant' (Roizman, 1971; Epstein & Achong, 1973). It is of interest that SMA and related patterns do not occur more frequently in the well established hereditary autoimmune disorders of the thyroiditis–gastritis–adrenalitis class. While doing the present investigation we evaluated the incidence in these diseases (Table 2) with the same

TABLE 2. Smooth muscle fluorescence in organ-specific autoimmune disease

Condition	Number of patients	Percentage of SMA positive*	Highest titre
Hashimoto & 1° myxoedema	154	7	20
Pernicious anaemia	85	13	20
Addison's disease	18	11	20

* Minimum titre 10; weak reactions were disregarded.

tissues and conjugates, and found no more SMA than in healthy controls. Conversely, organ-specific and other tissue antibodies which are present in high titres in autoimmune disorders, were practically absent in infectious mononucleosis, due to the young age of the patients and controls. Tissue antibodies other than SMA are a feature of increasing age, with female preponderance and familial clustering, while SMA is independent of sex and age and is not familial.

Reticulin antibodies are also independent of age and sex. They have been separated into at least five patterns (Rizzetto & Doniach, 1973), all of which are seen mainly in coeliac disease and other malabsorption syndromes. Their incidence was not increased in infectious mononucleosis or in hepatitis. Thus, although IM is characterized by a multiplicity of abnormal antibodies, this is by no means an unselected process. A temporary depression of delayed hypersensitivity has been demonstrated in IM (Haider *et al.*, 1973) and if Allison's theory (1973) of a positive refraining action of T cells over autoantibody synthesis applies to man, this transient T-cell dysfunction could account for the autoantibodies, although the curious selectivity remains unexplained. Another possibility is that the EB virus stimulates the

synthesis of antibodies directly in the clones of B cells it infects, and that the transformed T lymphocytes in the circulation represent an expanded clone of cells which help to terminate the infection as suggested by Steel & Ling (1973).

ACKNOWLEDGMENTS

We thank Granville and Marlene Swana and Gay Medley for their excellent technical assistance, and Miss Hilary Fischler for preparing the manuscript. Work in the Department of Immunology is supported by the Medical Research Council and the World Health Organization.

REFERENCES

AJDUKIEWICZ, A., DUDLEY, F., FOX, R.A., SHERLOCK, S. & DONIACH, D. (1972) Immunological studies in an epidemic of infective hepatitis. *Lancet*, **i**, 803.

ALLISON, A.C. (1973) Mechanisms of tolerance and autoimmunity. *Ann. rheum. Dis.* **32**, 283.

CARTER, R.L. (1966) Antibody formation in infectious mononucleosis. II. Other 19S antibodies and false-positive serology. *Brit. J. Haematol.* **12**, 268.

EPSTEIN, M.A. & ACHONG, B.G. (1973) Various forms of Epstein–Barr virus infection in man: established facts and a general concept. *Lancet*, **ii**, 836.

FISHER, R.A. & YATES, F. (1963) *Statistical Tables.* Oliver & Boyd, London.

HAIDER, S., COUTINHO, M.L., EMOND, R.T.D. & SUTTON, R.N.P. (1973) Tuberculin anergy and infectious mononucleosis. *Lancet*, **ii**, 74.

HOLBOROW, E.J. (1972a) Smooth muscle antibodies in viral hepatitis. *Brit. med. Bull.* **28**, 142.

HOLBOROW, E.J. (1972b) Smooth muscle antibodies and antigens. *Proc. R. Soc. B.* **65**, 481.

HOLBOROW, E.J., ASHERSON, G.L., JOHNSON, G.D., BARNES, R.D.S. & CARMICHAEL, D.L. (1963) Antinuclear factor and other antibodies in blood and liver diseases. *Brit. med. J.* **1**, 656.

HOLBOROW, E.J., HEMSTED, E.H. & MEAD, S.V. (1973) Smooth muscle antibodies in infectious mononucleosis. *Brit. med. J.*, **3**, 323.

KAPLAN, M.E. (1968) Cryoglobulinaemia in infectious mononucleosis; quantitation and characterization of the cryoproteins. *J. Lab. clin. Med.* **71**, 754.

LANCET (1973) **ii**, 712. Editorial. Immunopathology of infectious mononucleosis.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235.

MOTTIRONI, V.D. & TERASAKI, P.J. (1970) Lymphocytotoxity in disease. I. Infectious mononucleosis, rubella and measles. *Histocompatibility Testing* (Ed. by P. I. Teresaki), p. 301. Munksgaard, Copenhagen.

RIZZETTO, M., SWANA, G. & DONIACH. (1973) Microsomal antibodies in active chronic hepatitis and other disorders. *Clin. exp. Immunol.* **15**, 331.

RIZZETTO, M. & DONIACH, D. (1973) Types of reticulin antibodies detected in human sera by IFL. *J. clin. Path.* **26**, 841.

ROIZMAN, B. (1971) Herpes viruses, membranes and the social behaviour of infected cells. *Viruses Affecting Man and Animals* (Ed. by M. Saunders & M. Schaeffer). W. H. Green, St. Louis, Missouri.

SEAH, P.P., FRY, L., HOLBOROW, E.J., ROSSITER, M.A., DOE, W.F., MAGALHAES, A.F. & HOFFBRAND, A.V. (1973) Antireticulin antibody: incidence and diagnostic significance. *Gut*, **14**, 311.

SIEGEL, S. (1956) *Non-parametric Statistics.* McGraw–Hill, New York.

STEEL, C.M. & LING, N.R. (1973) Immunopathology of infectious mononucleosis. *Lancet*, **ii**, 861.

SUTTON, R.N.P. (1972) The EB virus in relation to infectious mononucleosis. *J. clin. Path.* **25**, 58.

SUTTON, R.N.P., REYNOLDS, K., ALMOND, J.P., MARSTON, S.D. & EMOND, R.T.D. (1973) Immunoglobulins and EB virus antibodies in infectious mononucleosis. *Clin. exp. Immunol.* **13**, 359.

TERASAKI, P.J., MOTTIRONI, V.D. & BARNETT, E.V. (1970) Cytotoxins in disease; autotoxins in lupus. *N. Engl. J. Med.* **283**, 724.

THOMAS, D.B. (1972) Antibodies to membrane antigen common to thymocytes and a subpopulation of lymphocytes in infectious mononucleosis sera. *Lancet*, **i**, 399.

THOMAS, D.B. (1973) Antibodies specific for human T-lymphocytes in cold agglutinin and lymphocytotoxic sera. *Europ. J. Immunol.* **3,** 824.

THOMAS, D.B. & PHILLIPS, B. (1973) Membrane antigens specific for human lymphoid cells in the dividing phase. *J. exp. Med.* **138,** 64.

TRENCHEV, P., SNEYD, P. & HOLBOROW, E.J. (1974) Immunofluorescent tracing of smooth muscle contractile protein antigens in tissue other than smooth muscle. *Clin. exp. Immunol.* **16,** 125.

WHITEHOUSE, J.M.A., HOLBOROW, E.J. (1971) Smooth muscle antibody in malignant disease. *Brit. med. J.* **4,** 511.

WOLLHEIM, F.A. & WILLIAMS, R.C. (1966). Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M(IgM). Increases in infectious mononucleosis. *N. Engl. J. Med.* **274,** 61.