Clin. exp. Immunol. (1987) **68**, 562–569

Antibody affinity and IgG subclass of responses to tetanus toxoid in patients with rheumatoid arthritis and systemic lupus erythematosus

MADELEINE E. DEVEY, KATHARINE BLEASDALE & D. A. ISENBERG*

*Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London WC1 and *Bloomsbury Rheumatology Department, UCH/Middlesex Hospitals, Arthur Stanley House, London W1*

(Accepted for publication 23 December 1986)

SUMMARY

Significant differences in both the affinity and IgG subclass of antibodies produced after immunization with tetanus toxoid have been demonstrated in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) compared to healthy controls. Patients with RA failed to show affinity maturation although they produced similar amounts of antibody to the controls. Some patients with SLE produced very high affinity antibodies although there was a wide spectrum of response. Antibodies to tetanus toxoid in controls were predominantly IgG1 and IgG4 but in RA and SLE there was either a restricted IgG1 response or a more general response in all the IgG subclasses. It is likely that these differences in response reflect the underlying disorders in immunoregulation present in patients with these diseases.

Keywords antibody affinity IgG subclass RA SLE tetanus toxoid

INTRODUCTION

Experimental evidence from a number of studies shows that high affinity antibodies mediate a variety of biological functions more effectively than low affinity antibodies (Steward, 1981). Thus, the importance of characterizing antibody responses in terms of affinity, as well as amount, for example after vaccination, has been stressed (Brown *et al.*, 1984). Antibody affinity is a genetically controlled parameter of the immune response governed by mechanisms independent of those regulating antibody levels. It has been argued that the inability to produce high affinity antibodies is a form of immunodeficiency which may have immunopathological consequences (Soothill & Steward, 1971). This has been confirmed experimentally in mice, genetically selected on the basis of the affinity of their antibody responses, in which it has been shown that low affinity responses and, in particular, failure of affinity maturation, are important in determining susceptibility to chronic immune complex disease (Devey *et al.*, 1984).

Based on the premise that the control of antibody affinity is broadly antigen independent and is thus likely to reflect overall immune responsiveness (Soothill & Steward, 1971), we have determined affinity responses in patients with SLE and RA after immunization with tetanus toxoid. In view of the importance of assessing the affinity as well as the amount of antibodies produced in response to vaccination (Brown *et al.*, 1984), information on the affinity responses in such patients may have important implications for their protection after immunization. In addition, we have examined the

Correspondence: Dr M. E. Devey, Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.

IgG subclass of antibody responses to tetanus toxoid, as previous studies (Devey *et al.*, 1985) suggest that there is an association between antibody affinity and IgG subclass in man.

MATERIALS AND METHODS

Human sera. Sera were obtained from 24 patients with SLE (mean age 40·5 years, range 20–79), each of whom met four or more of the American Rheumatism Association's revised criteria for the classification of the disease (Tan *et al.*, 1982); 29 patients with RA (mean age 53·8 years, range 23–73) each of whom had definite or classical disease according to the criteria of the American Rheumatism Association (Ropes *et al.*, 1958); and 33 healthy controls (mean age 36·6 years, range 22–79), before and 2–3 weeks after intramuscular immunization with 40 IU alum-adsorbed tetanus toxoid (Wellcome). A second immunization was given 4–6 weeks after the first, if sufficient antibody was not obtained. Patients receiving immunosuppressive drugs, except for low dose prednisolone (< 15 mg/day), were not included in the study.

Affinity assay. Antibody affinity was measured by a double-isotope radioimmunoprecipitation assay, modified from Gaze, West & Steward (1973) using polyethylene glycol (mol. wt 8000) at a final concentration of 3·0% to separate bound antigen (b) from free antigen (c). Antibody affinity (K) and antibody concentration, expressed as total antibody binding sites (Abt), were calculated from a plot of $1/b$ versus $1/c$ in a modification of the Langmuir equation (Steward & Petty, 1972).

Antisera. Monoclonal antibodies to IgG1 (NL16), IgG2 (GOM1), IgG3 (ZG4) and IgG4 (GB7B) were obtained from Seward Laboratories and used at dilutions (1/2500, 1/5000, 1/5000 and 1/5000, respectively) which had been shown to have equivalent activity with the appropriate purified subclass protein in ELISA. An affinity purified, human IgG absorbed, peroxidase-conjugated antiserum to mouse IgG (Jackson) was used at 1/5000.

Enzyme-linked immunosorbent assay. Polystyrene microtitre plates (Flow Laboratories) were coated with affinity purified tetanus toxoid (10 μg/ml) in 0·1 M carbonate buffer, pH 9·6. Doubling dilutions of sera were made from a starting dilution of 1/20 in PBS/1% casein/0·05% Tween 20 (PBS/C/T). Plates were incubated with monoclonal anti-subclass antibodies followed by the peroxidase conjugate. The plates were washed between each step with PBS/C/T and all incubations were for 2 h at room temperature. Finally, 0·4 mg/ml *o*-phenylene diamine dihydrochloride was added to each well, the reaction stopped after 10 min and the resulting colour measured at 492 nm in a Titertek Multiskan. Positive and negative controls were included on each plate and the results were expressed as an end point titre at a low optical density ($OD_{492} = 0{\cdot}2$).

Statistics. Statistical analysis of data was carried out using Student's *t*-test, the Wilcoxon two-sample test for non-parametric data and regression analysis.

RESULTS

Antibody levels. Antibodies to tetanus toxoid were detected in 73% controls, 54% SLE patients and 59% RA patients before immunization and a significant increase in the mean antibody level (Abt) occurred in all groups after immunization (Fig. 1) with no significant differences between the groups. Immunization was not successful in two controls, one patient with RA and one with SLE.

Antibody affinity. Pre-immunization antibody was of low affinity in all groups and a significant increase in affinity occurred in controls ($P < 0{\cdot}0001$) and SLE ($P < 0{\cdot}05$) after immunization (Fig. 2). RA patients did not show this affinity maturation and had significantly lower affinity values compared to both the controls and SLE patients. Antibody affinity showed a significantly inverse correlation with age in the controls ($r = 0{\cdot}44$, $P = 0{\cdot}014$), but not in RA or SLE. In addition, affinity in RA patients over the age of 50 ($0{\cdot}66 + 0{\cdot}51 \times 10^6$ M^{-1}) was not significantly different from that in patients under the age of 50 ($0{\cdot}65 + 0{\cdot}41 \times 10^6$ M^{-1}).

SLE patients showed a very wide variation in affinity responses after immunization, 39% produced antibody of very high affinity ($> 2{\cdot}0 \times 10^6$ M^{-1}) compared to 15% of controls with values in this range. There was no correlation between either high or low affinity responses and any of a

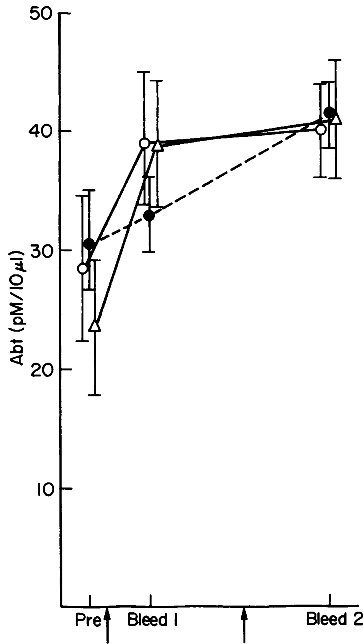

Fig. 1. Total antibody levels (Abt, pM/10 μl ± s.e.) in SLE (△—△), RA (○—○) and normal controls (●- - -●) before (Pre) and after immunization with tetanus toxoid (arrow).

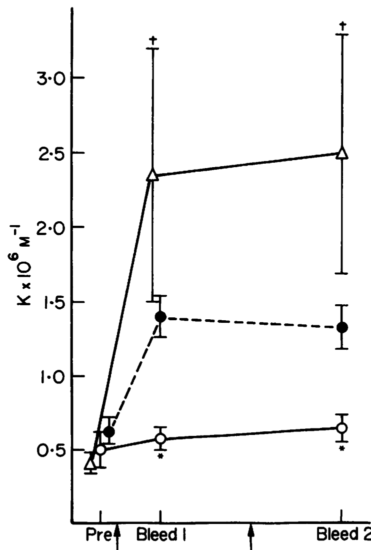

Fig. 2. Antibody affinity (K, $\times 10^6 M^{-1} \pm$ s.e.) in SLE (△—△), RA (○—○) and normal controls (●- - -●) before (Pre) and after immunization with tetanus toxoid (arrow). Significant differences between RA and controls, * $P<0.0001$ and between SLE and RA, † $P<0.05$.

Table 1. Antibody affinity after immunisation with tetanus toxoid and clinical manifestations of disease in patients with SLE

Clinical feature*		n	Antibody affinity $\times 10^6\ \text{M}^{-1} \pm$ s.d.
Rash	+	17	2·51 ± 4·52
	−	6	2·42 ± 1·45
Raynaud's	+	11	2·00 ± 2·40
	−	12	2·94 ± 5·00
Serositis	+	13	2·76 ± 4·85
	−	10	2·13 ± 2·42
Renal disease	+	2	0·66 ± 0·08
	−	21	2·66 ± 4·06
CNS disease	+	6	1·38 ± 1·09
	−	17	2·88 ± 4·49
DNA antibodies	+	10	2·95 ± 5·50
	−	13	2·13 ± 2·26
ENA antibodies	+	12	2·48 ± 2·37
	−	11	2·49 ± 5·25

* All the patients tested had arthralgia/arthritis. There were also no differences between those with Sjögren's syndrome or other autoimmune diseases (data not shown).

Table 2. Antibody affinity after immunization with tetanus toxoid in patients with SLE and RA treated with prednisolone and non-steroidal anti-inflammatory drugs (NSAID)

Patients	Therapy	n	Antibody affinity $\times 10^6\ \text{M}^{-1} \pm$ s.d.
SLE	Prednisolone	9	4·57 ± 5·68*
	Other†	12	1·11 ± 0·91
RA	NSAID	12	0·76 ± 0·62
	Prednisolone	3	0·56 ± 0·23
	Other†	11	0·55 ± 0·31
Controls	−	28	1·33 ± 0·82

* Significant difference between prednisolone treatment and 'other' therapy, $P < 0{\cdot}05$.

† No therapy or other therapy excluding NSAID and prednisolone.

wide variety of classical features of lupus that were analysed. These included skin rash, Raynaud's phenomenon, arthralgia, pleuropericarditis, renal disease (two patients only), cerebral disease, the presence or absence of Sjögren's syndrome and other autoimmune diseases (Table 1). However, there was a significant association between high affinity responses in SLE patients and low dose prednisolone therapy (Table 2). This was not seen in the small number of RA patients receiving prednisolone, in whom the highest mean affinity was seen in patients treated with a variety of non-steroidal anti-inflammatory drugs, although the difference was not significant (Table 2).

IgG subclass of antibody to tetanus toxoid. The predominant antibody response to tetanus toxoid in normal individuals was in the IgG1 and IgG4 subclasses (Fig. 3). Before immunization, 41·2%

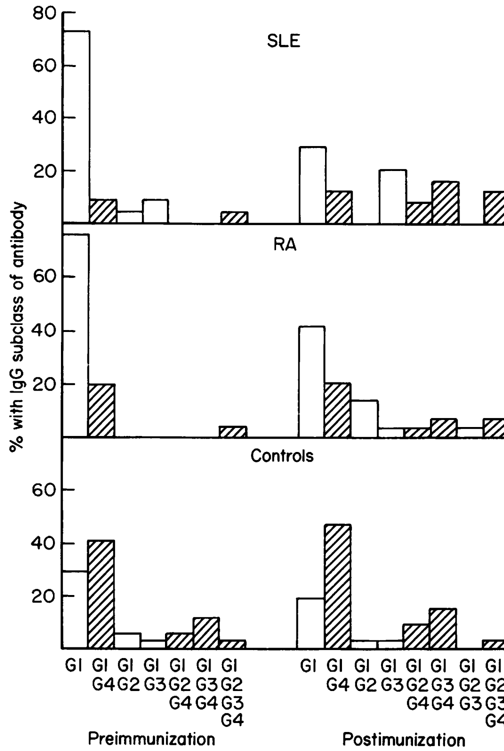

Fig. 3. IgG subclass distribution of antibodies to tetanus toxoid in SLE, RA and normal controls before and after immunization with tetanus toxoid (cross-hatching indicates the presence of IgG4 antibodies).

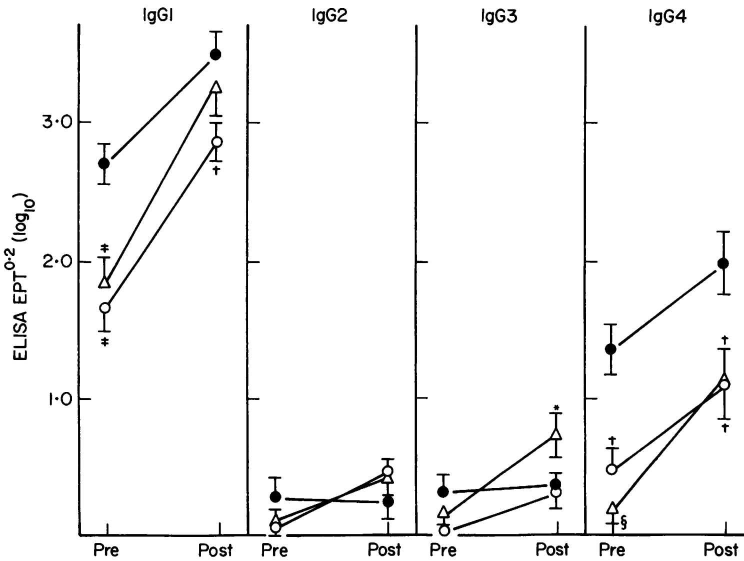

Fig. 4. The relative levels of antibodies to tetanus toxoid in SLE (△—△), RA (○—○) and normal controls (●—●) before and after immunization with tetanus toxoid. Significant differences between patients and controls, * $P<0·05$, † $P<0·01$, ‡ $P<0·001$, § $P<0·0001$.

Table 3. Affinity and amount of antibodies to tetanus toxoid in patients and controls with high IgG4 antibody levels relative to IgG1 (IgG4 > IgG1) and high IgG1 antibody levels relative to IgG4 (IgG1 > IgG4)

	Subclass	*n*	Antibody affinity $\times 10^6$ M^{-1} $\pm$ s.d.	Antibody level pM/10 μl $\pm$ s.d.
Controls	IgG4 > IgG1	10	0·91 ± 0·20*	35·11 ± 8·57
	IgG1 > IgG4	17	1·72 ± 0·76	43·36 ± 18·45
RA	IgG4 > IgG1	5	0·64 ± 0·14	36·66 ± 14·67
	IgG1 > IgG4	22	0·67 ± 0·51	40·50 ± 24·31
SLE	IgG4 > IgG1	1	0·98	22·1
	IgG1 > IgG4	22	2·54 ± 3·97	41·28 ± 20·98

* Significant difference between IgG4 > IgG1 and IgG1 > IgG4, $P < 0{\cdot}005$.

controls had antibody of both isotypes and 29·4% had IgG1 antibody alone. After boosting, there was a small shift from IgG1 alone to the IgG1–IgG4 antibody response. In both SLE and RA, pre-immunization, antibodies were highly restricted to IgG1 (72·7% and 76% respectively). After immunization, IgG1 remained the predominant isotype in both groups, followed by IgG1–IgG4 responses in RA (20·7%) and IgG1–IgG3 responses in SLE (20·8%). Although there was a general increase in antibodies in all four subclasses in SLE and, to a lesser extent, in RA, the highly restricted IgG1–IgG4 responses which predominated in the controls were not found (Fig. 3).

The relative amounts of antibody produced in the different subclasses were assessed by taking ELISA end-point titre at $OD_{492} = 0{\cdot}2$, which should reflect antibody concentration rather than affinity (Steward & Lew, 1985). There were significant increases in the levels of both IgG1 and IgG4 antibodies after immunization in SLE, RA and controls (Fig. 4). However, IgG4 antibody levels were significantly higher in controls compared to both SLE and RA before and after immunization. After immunization, small but significant increases in IgG2 antibodies occurred in both SLE and RA. In addition, there was also a significant increase in IgG3 antibody levels in SLE.

IgG subclass and affinity. Antibody affinity was significantly lower in controls with high IgG4 antibody levels relative to IgG1 antibody levels (Table 3). This association was not seen in RA. No conclusions could be made in SLE as only one patient had high levels of IgG4 antibody.

DISCUSSION

Although there is clear evidence of abnormal immune regulation in SLE at the cellular level, humoral responses to a variety of exogenous antigens have often been found to be normal (Mitchell *et al.*, 1982) and similar findings have been reported in RA (reviewed by Yu & Peter, 1974). In this study the total amount of antibody produced after immunization with tetanus toxoid was found to be similar in patients with SLE and RA compared to normal controls. However, several major differences were found when antibody affinity and IgG subclass responses were compared. Patients with RA failed to show affinity maturation after immunization and, although their mean age was higher than the other groups, there was no correlation between age and affinity responses. On the other hand, affinity maturation occurred in SLE but the range of antibody affinity was very wide, analogous to the abnormal (very high or very low) antibody responses described *in vitro* in SLE after influenza immunization (Mitchell *et al.*, 1982). There were no significant correlations between affinity responses in SLE and a wide variety of classical features of lupus, although affinity was low in two patients with renal disease consistent with reported low avidity anti-DNA antibodies in renal SLE (Steward, 1976). However, there was a significant correlation between high affinity antibody responses in SLE and low dose prednisolone therapy. This may be related to the effects of glucocorticoids on arachadonic acid metabolism (Hirata *et al.*, 1980), which may affect a broad range of immune functions and this finding requires further investigation.

There have been a number of studies on subclass restriction of antibodies to tetanus toxoid (reviewed by Hammarstrom & Smith, 1986) and most suggest that responses in normal individuals are predominantly found in the IgG1 and IgG4 subclasses. This was confirmed here, where antibodies in normal controls were found to be IgG1 and IgG4 (46·9%) or IgG1 alone (18·8%). The IgG subclass distribution of antibodies to tetanus toxoid in both SLE and RA was quite different from that in the controls, with a highly restricted IgG1 responses before immunization and a more general increase in antibodies in all subclasses after immunization and, notably in SLE, in the complement fixing IgG3 subclass.

The previously reported association between high levels of IgG4 antibodies and low affinity responses to tetanus toxoid (Devey *et al.*, 1985) was confirmed in the controls in this study, although it was not found not in patients with RA. This raises the question as to whether IgG4 antibodies are intrinsically of lower affinity compared to IgG1, or whether antibodies of both isotypes are low affinity in individuals with high levels of IgG4 antibody. Preliminary results have shown that the dissociability of IgG4 antibodies for tetanus toxoid is greater than that of IgG1 antibodies in the same serum, indicating that IgG4 is of lower functional affinity (M.E. Devey, K. Bleasdale & S. Rath, unpublished). Similar affinity differences have been described for the murine IgG subclasses (Sarvas *et al.*, 1983), suggesting that there may be a link between affinity maturation and IgG subclass expression.

Analysis of IgG subclass deficiencies in man have shown an association with heavy chain constant region gene order, being more frequent for the subclasses with C_H genes located downstream (3^1) on chromosome 14 ($\gamma2$ and $\gamma4$) compared to those with genes located upstream ($\gamma1$ and $\gamma3$). What controls isotype switching is not well understood but it has been proposed that T cells are involved (Mayumi *et al.*, 1983; Mayer, Posnett & Kunkel, 1985). Selective deficiencies of IgG subclasses have been demonstrated in many diseases including RA (Shakib & Stanworth, 1980) and SLE (Oxelius, 1984), both of which may be associated with elevated levels of IgG1 and low levels of IgG2 and IgG4. There is less information on IgG subclass restriction of antibody responses in these diseases although it has been proposed that a switch from complement fixing (IgG1 and IgG3) to non-complement fixing (IgG2 and IgG4) autoantibodies may result in clinical improvement in patients with autoimmune diseases (Tojo, Friou & Spiegelberg, 1970). What emerges from this study, is that deviations from normal antibody responses to tetanus toxoid, in terms of subclass restriction, occur in both SLE and RA although whether this is a reflection of subclass restriction of autoantibodies in these patients is not known at present.

It is clear that patients with SLE and RA also exhibit abnormal immune responses with regard to the affinity of their antibody responses to tetanus toxoid, when compared to healthy controls. Differences were also apparent within the two patient groups. For example, patients with RA were unable to show affinity maturation, whereas patients with SLE either had very high or very low affinity responses. This finding may have important implications for the protection of RA patients against tetanus and also suggests that their reponses to other immunizing antigens may be impaired. It is likely that both the regulation of antibody affinity and IgG subclass expression are dependent on cellular control, possibly at the level of the T cell, and therefore the abnormalities described here may reflect the underlying disorders of immunoregulation in these diseases.

We thank Dr M. Snaith for supporting the project and helping to collect the blood samples, Professor M. W. Steward for helpful criticism of the manuscript, Wellcome Biotechnology Ltd for the affinity purified tetanus toxoid and the Wellcome Trust for financial support. M.E.D. is a Wellcome Trust senior lecturer.

REFERENCES

Brown, S.E., Howard, C.R., Zuckerman, A.J. & Steward, M.W. (1984) Affinity of antibody responses in man to hepatitis B vaccine determined with synthetic peptides. *Lancet* **ii,** 184.

Devey, M.E., Bleasdale, K., Stanley, C. & Steward, M.W. (1984) Failure of affinity maturation leads to increased susceptibility to immune complex glomerulonephritis. *Immunology* **52,** 377.

Devey, M.E., Bleasdale, K.M., French, M.A.H. & Harrison, G. (1985) The IgG4 subclass is associated with a low affinity antibody response to tetanus toxoid in man. *Immunology* **55,** 565.

GAZE, S., WEST, N.J. & STEWARD, M.W. (1973) The use of a double isotope method in the determination of antibody affinity. *J. immunol. Methods* **3,** 357.

HAMMARSTROM, L. & SMITH, C.I.E. (1986) IgG subclass changes in response to vaccination. *Monogr. Allergy* **19,** 241.

HIRATA, F., SCHIFFMANN, E., VENKATASUBRAMANIAN, K., SALOMON, D. & AXELROD, J. (1980) A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. natn Acad. Sci. USA,* **77,** 2533.

MAYER, L., POSNETT, D.N. & KUNKEL, H.G. (1985) Human malignant T cells capable of inducing an immunoglobulin class switch. *J. exp. Med.* **161,** 134.

MAYUMI, M., KURITANI, T., KUBAGAWA, H. & COOPER, M.D. (1982) IgG subclass expression by human B lymphocytes and plasma cells: B lymphocytes precommitted to IgG subclass can be preferentially induced by polyclonal mitogens with T cell help. *J. Immunol.* **130,** 671.

MITCHELL, D.M., FITZHARRIS, P., KNIGHT, R.A., SCHILD, G.C. & SNAITH, M.L. (1982) Kinetics of specific anti-influenza antibody production by cultured lymphocytes from patients with systemic lupus erythematosus following influenza immunization. *Clin. exp. Immunol.* **49,** 290.

OXELIUS, V. (1984) Immunoglobulin G (IgG) subclasses and human disease. *Am. J. Med.* **76,** 7.

ROPES, M.W., BENNETT, G.A., COBB, S., JACOBS, R. & JESSON, R.A. (1958) Revision of the diagnostic criteria for rheumatoid arthritis. *Bull. rheum. Dis.* **9,** 175.

SARVAS, H.O., SEPPALA, I.J.T., TAHTINEN, T., PETERFY, F. & MAKELA, O. (1983) Mouse IgG antibodies have subclass associated affinity differences. *Mol. Immunol.* **20,** 239.

SHAKIB, F. & STANWORTH, D.R. (1980) Human IgG subclasses in health and disease, Part II. *La Ricerca Clin. Lab.* **10,** 561.

SOOTHILL, J.F. & STEWARD, M.W. (1971) The immunopathological significance of the heterogeneity of antibody affinity. *Clin. exp. Immunol.* **9,** 193.

STEWARD, M.W. (1976) The role of low-affinity antibody in immune complex disease. In: *Infection and Immunology in the Rheumatic Diseases* (ed. D.C. Dumonde), Chap. 55, Blackwell, Oxford.

STEWARD, M.W. (1981) The biological significance of antibody affinity. *Immunology Today* **2,** 134.

STEWARD, M.W. & LEW, A.M. (1985) The importance of antibody affinity in the performance of immunoassays for antibody. *J. Immunol. Methods* **78,** 173.

STEWARD M.W. & PETTY R.E. (1972) The use of ammonium sulphate globulin precipitation for determination of affinity of anti-protein antibodies in mouse serum. *Immunology* **22,** 747.

TAN, E.M., COHEN, A.J., FRIES, J.F., MASI, A.J., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALD, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25,** 1281.

TOJO, T., FRIOU, G.J. & SPIEGELBERG, H.L. (1970) Immunoglobulin G subclass of human anti-nuclear antibodies. *Clin. exp. Immunol.* **6,** 145.

YU, D.T.Y. & PETER, J.B. (1974) Cellular immunological aspects of rheumatoid arthritis. *Sem. Arth. Rheum.* **4,** 25.