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Impaired IgG response to tetanus toxoid in human membranous nephropathy: association with HLA-DR3

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SUMMARY

The IgG response to tetanus toxoid (TT) immunization was quantitated by radioimmunoassay in patients with membranous nephropathy (MN) and healthy controls. Variation in subclass (ELISA) and electrical charge (isoelectric focussing, immunofixation & autoradiography) of the IgG response were also assessed. Total IgG and IgG subclass responses were impaired in MN compared to controls, although this was only significant for IgG-3 ($P < 0{\cdot}05$). Non responders to TT were more common in MN, and response was independent of disease activity. No distinctive pattern of IgG subclass response or IgG spectrotype was seen in MN. Impaired response to TT was associated with HLA-DR3 among controls, and in MN (88·8% of whom were DR3) markedly depressed responses occurred in apparent DR3 homozygotes.

Keywords membranous nephropathy tetanus toxoid HLA-DR antigens IgG subclass

INTRODUCTION

IgG is the characteristic immunoglobulin deposited in the glomerulus in human membranous nephropathy (MN). It was previously thought that the granular deposits of IgG seen along the basement membrane on immunofluorescent microscopy (and the subepithelial electron dense deposits on electron microscopy to which they correspond) represented the antibody component of circulating antigen-antibody complexes which had been passively trapped in the glomerulus. However much recent evidence suggests that complexes are formed *in situ* by the accretion of antibody to antigen (exogenous or endogenous) already fixed to the basement membrane (Couser & Salant, 1980). The nature of this antigen in the great majority of MN remains unknown.

Physiochemical variations in IgG may be important in determining the development of the MN lesion. Semiquantitative immunofluorescence microscopy suggests that the proportion of IgG-3 and IgG-4 is higher in glomerular deposits in MN than in normal serum (Bannister *et al.*, 1983). Animal work has shown in MN than in normal electrical charge, since cationic proteins (including IgG) more easily deposit in the subepithelial site (Batsford, Takamiya & Vogt, 1980; Oite *et al.*, 1982).

There is strong evidence that predisposition to MN has an immunogenetic basis, since greater than 70% of adults with MN are HLA-DR3 positive (Klouda, Manos & Acheson, 1979).

In this study the IgG response to immunization with tetanus toxoid (TT) was examined in

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patients with MN and in healthy controls. The total IgG response was quantitated and subclass and charge variations assessed. The response was analysed in the context of the clinical state at the time of study and the HLA type.

MATERIALS AND METHODS

Patients with MN. Twenty patients with MN were studied (16 male, 4 female, mean age 53·4 ± 3·3 years). All had presented with a nephrotic syndrome (mean interval since diagnosis 9·1 ± 1·1 years) and had renal appearances characteristic of MN. None had evidence of malignancy or SLE and none had been exposed to nephrotoxins known to induce MN.

At the time of study 10 patients were in remission (defined as proteinuria $<0{\cdot}5$ g/24 h) with stable renal function (serum creatinine (SCr) 94 ± 3 μmol/l; creatinine clearance (CCr) 90 ± 5 ml/min/1·73 m^2). Ten patients had persistent proteinuria (8·2 ± 86 μmol/l, CCr 60 ± 15 ml/min/1·73 m^2) and four patients had moderate to severe renal impairment (SCr 306–843 μmol/l; CCr 6–22 ml/min/1·73 m^2). Five patients had received corticosteroids in the past (duration of treatment 1–3 years) and the intervals since steroids had been given are 1, 5, 13, 15 and 17 years.

Controls. Twenty healthy subjects (10 male, 10 female, mean age 38·7 ± 7·8 years) were studied as controls. All patients and controls had received TT in the past without adverse effects.

Methods. Each subject completed a 24 h urine collection and venous blood was taken before the intramuscular injection of 0·5 ml adsorbed tetanus toxoid (Duncan Flockhart, Greenford, Middlesex, UK). A further blood sample was taken 3 weeks later. Serum was separated and stored at −40°C until assayed. Urinary protein, serum albumin, serum creatinine and creatinine clearance were determined by routine laboratory techniques. The total TT specific IgG response was measured by radioimmunoassay (RIA) and subclass responses by enzyme-linked immunosorbent assay (ELISA). The charge spectrum of the IgG response was assessed by isoelectric focussing (IEF), immunofixation and autoradiography. Each subject was typed for HLA-A, -B and -DR.

RIA for IgG antibodies to tetanus toxoid (IgG-TT). A solid phase sandwich RIA was used. Multiwell plates with removable wells (disposawells, Dynatech, Billingshurst, West Sussex, UK) were coated by incubating for 18 h at 4°C with a 1:50 dilution of tetanus toxoid (Wellcome Reagents Ltd., Beckenham, UK) in carbonate-bicarbonate buffer pH 9·6. Wells were washed and 'blocked' (to minimise non-specific protein binding) by incubating for 1 h with BSA 1 mg/ml. Duplicates of test sera at five different dilutions (1:15 in three-fold dilutions to 1:4050 in PBS-BSA-Tween-Azide buffer) were incubated in the wells. A standard curve was included in every assay based on human anti-tetanus immunoglobulin (Blood Product Laboratories, Elstree, Hertfordshire, UK) in two-fold dilutions from 1:800 to 1:409,600. After overnight incubation the wells were washed thoroughly and 200 μl of radiolabelled anti-human IgG was added at a dilution giving 20–30,000 ct/min. (Anti-human IgG (Dako Ltd., Buckinghamshire, UK) was affinity purified and labelled with iodine-125 by the chloramine T method (Hunter & Greenwood 1962).) The wells were washed and bound radioactivity counted on a gamma counter (Wallac 1270 Tackgamma II, LKB, Stockholm, Sweden). Results of unknowns were expressed in units equivalent to mIU/ml of standard curve human anti-tetanus IgG.

Antitetanus IgG subclass assays (IgG1-4-TT). A solid phase sandwich assay was used. Flat bottomed multiwell plates (Dynatch) were coated with simple TT by incubating at 4°C for 18 h in 0·1 M sodium carbonate-bicarbonate buffer pH 9·6. After washing with PBS-Tween the plates were 'blocked' (to minimise non-specific protein adsorption) by incubating for 1 h with BSA 1 mg/ml. Dilutions of the test serum were made in PBS-BSA-Tween buffer containing 0·01% sodium azide and incubated at 4°C for 18 h. After washing, wells were incubated for 4 h with anti-IgG subclass monoclonal antibodies (Unipath Ltd., Bedford, UK) diluted 1:1000. After further washing, peroxidase-conjugated antimouse Ig (Dako Ltd., Buckinghamshire, UK), diluted 1:1000 in azide free buffer was added at 4°C for 4 h. After thorough washing plates were 'developed' by the addition of 2,2′-Azino-di (3-ethyl-benzthiazoline) sulfonic acid (ABTS) (Sigma Chemical Co., St Louis, Missouri, USA) in citrate phosphate buffer pH 5·0 with 0·002% hydrogen peroxide. Intensity of colour was read as absorbancy by an automatic plate reader (Multiscan, Flow Laboratories,

Hertfordshire, UK) using a 420 nm filter. The plate reader was linked to a PET micro-computer allowing efficient storage and manipulation of data.

Isoelectric focussing. Isoelectric focussing (IEF) of serum proteins was carried out in thin layer (0·5 mm) agarose gels containing wide range ampholines (pH 3·5–9·5) (LKB). IEF was performed at 10 C on an LKB multiphor system. One microlitre of test serum was placed directly on the gel near the anode and focussed for 1 h to a final voltage of 1·5 kV. Marker proteins of known isoelectric point (BDH Chemicals Ltd., Poole, Dorset, UK) were also focussed to establish the pH gradients of the gels.

Immunofixation. Cellulose acetate paper (Sepraphore III, Gelman) was thoroughly soaked in a 1:4 solution of rabbit antihuman IgG (Dako) which was placed on the gel immediately after focussing and left for 1 h. Gels were then washed in PBS followed by distilled water, air dried, and stained with silver (Willoughby & Lambert, 1983).

Autoradiography. Autoradiography was used to establish the isoelectric points of IgG directed against antigenic determinants of tetanus toxoid. Tetanus toxoid (Wellcome Reagents Ltd., Beckenham, IK) was labelled with iodine-125 by the chloramine T method (Hunter & Greenwood, 1962). The labelled protein was dialysed against PBS for 48 h to minimise unbound radioactivity. Test serum was isoelectric focussed and immunofixed with anti-IgG as described above. After immunofixation, the gels were washed overnight and then overlaid with cellulose acetate paper (Sepraphore III, Gelman) soaked in a 1:50 solution of the dialysed radiolabelled TT. After 18 h the paper was removed, the gel washed thoroughly for 72 h in PBS followed by distilled water, and air dried.

In a dark room, the gels were placed next to a radiographic film (Kodak-X-omat xar-5) and held immobile between glass plates. They were stored with total light exclusion for 6 days. The radiographs were then developed in an automatic film processor. The immunofixed gels were silver stained.

HLA typing. Individuals were tested for 17 HLA-A and 23 HLA-B antigens using the standard National Institutes of Health lymphocytoxicity technique. HLA-DR typing for antigens 1–8 was carried out on peripheral blood B lymphocytes isolated by their adherence to nylon wool columns (Danilovs, Ayoub & Terasaki, 1980).

All sera used for HLA typing were either obtained locally or were donated by established tissue typing laboratories through mutual exchange. The antisera were thoroughly characterized using a local cell panel which had been typed using international histocompatibility workshop antisera.

Statistical methods. Non-parametric ranked data were compared by the Mann-Whitney U test, and correlations sought using Kendal's tau test. Comparison of frequencies used Fisher's exact test and chi-squared test as appropriate.

RESULTS

Total IgG and IgG subclass response to TT

Controls (38·7 $\pm$ 7·8 years) were younger than MN subjects (53·4 $\pm$ 3·3 years). However there was no correlation of age with IgG-TT response in controls or MN (Kendall's tau test).

Initial IgG-TT was significantly less in MN than controls (Table 1; $P < 0{\cdot}02$). Pre-immunization IgG-TT was not closely correlated with serum total IgG ($r = 0{\cdot}14$) or amount of proteinuria ($r = 0{\cdot}14$). After TT was administered median responses of total IgG-TT and IgG subclasses were lower in MN than controls (Figs 1 & 2), although the difference is only significant for IgG-3 ($P < 0{\cdot}05$, Mann Whitney). However there were more non-responders (defined as post-immunization IgG-TT < 10 miu/ml) in MN (6/20) than controls (2/20). Among responders post-immunization IgG-TT was not significantly different in MN (318 (63-475) miu/ml) compared to controls (261 (94–627) miu/ml).

Subclass responses are in arbitrary units based on a response of 1,000 units in pooled human anti-TT immunoglobulin so quantitative comparison between different subclasses is not possible. However, comparison by ranking suggested that most individuals had comparable responses in all subclasses. There were occasional exceptions with individuals showing subclass variation in both MN and controls, but these fitted no pattern.

Table 1. IgG response to tetanus toxoid in MN and controls

	Controls ($n=20$)	MN ($n=20$)	
Pre-immunization TT-IgG (miu/ml)	18·3 (8·8–42·8)	4·5 (3·3–12·9)	$P<0·02$
Post-immunization TT-IgG* (miu/ml)	101 (4·3–350)	54·7 (5·8–368)	ns
IgG-1 (units)	32·3 (11·5–54·4)	13·2 (20–31)	ns
IgG-2 (units)	74·1 (18·1–109)	32·2 (8·9–57·7)	ns
IgG-3 (units)	44·1 (4·8–52·9)	11·3 (3·0–26·0)	$P<0·05$
IgG-4 (units)	45·7 (34·6–49·7)	37·3 (3·0–76·9)	ns

* Corrected for pre-TT-IgG by subtraction.
ns = Not significant

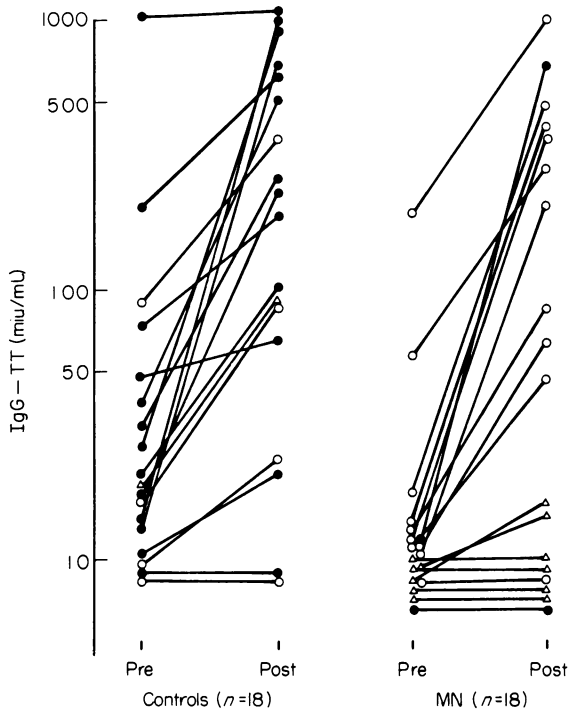

Fig. 1. Total tetanus toxoid specific IgG before and after immunization in MN and controls. (○) DR3; (△) DR3 homozygotes; (●) non DR3.

There were more female controls (10/20) than MN subjects (4/20), but within the control group total IgG-TT and subclass responses were not significantly different in females compared to males (data not shown).

To explain the tendency for those with MN to be non-responders to TT, associations were sought between IgG-TT and clinical and biochemical parameters within the MN group. However, there were no significant associations between IgG-TT and any of the following parameters: (a) degree of proteinuria, (b) serum albumin, (c) serum total IgG, (d) serum creatinine, (e) creatinine clearance, (f) time since diagnosis and (g) past use of corticosteroids.

Charge of the IgG response

The serum IgG spectrotype from each subject before and after TT was examined by IEF and

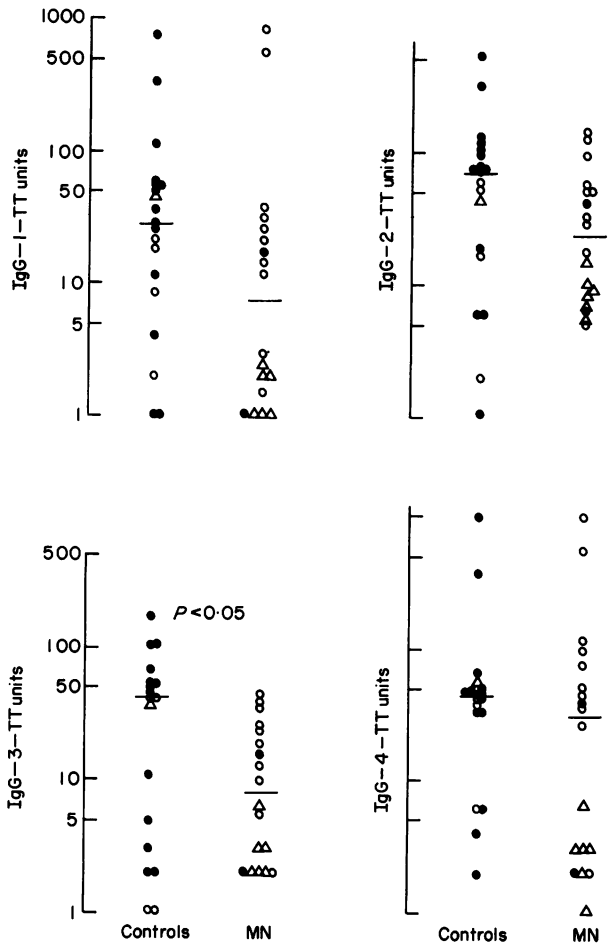

Fig. 2. IgG subclass responses to tetanus toxoid in MN and controls. (○) DR3; (△) DR3 homozygotes; (●) non DR3.

immunofixation (detecting IgG only). In each individual the spectrotype was constant, and comparison showed no new IgG bands appearing after TT in MN or controls.

In order to increase the sensitivity and specificity of the technique, allowing detection of TT-

Table 2. Association of DR3 with low TT response in controls

	Response of DR3 positive controls ($n=5$)		
	Submedian	Supramedian	Fisher
Total IgG	4/5	1/5	ns
IgG-1	4/5	1/5	ns
IgG-2	5/5	0/5	$P=0{\cdot}016$
IgG-3	4/5	1/5	ns
IgG-4	3/5	2/5	ns

ns = Not significant.

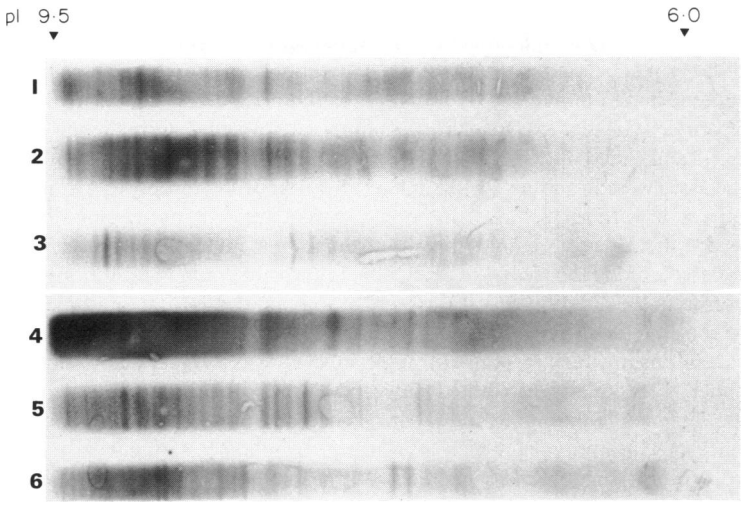

Fig. 3. Autoradiography (following isoelectric focussing and immunofixation of tetanus toxoid specific IgG in post-immunization serum of three subjects with MN (1–3) and three controls (4–6). In all cases there are IgG responses to multiple idiotypes with a wide range of electrical charge (pI 6·0–9·5), and charge spectrotype is similar in MN and controls.

specific IgG only, autoradiography was used. Figure 3 shows an autoradiograph of the TT-specific IgG in the post-immunization serum of three representative subjects with MN and three controls, all of whom had a significant response to TT. There are multiple banding patterns across the whole charge spectrum of IgG (pI 6·0–9·5). These patterns are consistent with response in all four subclasses and confirm the complex polyvalent nature of the antigen challenge given by TT. A similar range of IgG charge is seen in MN and controls.

HLA and the TT response

The distribution of DR antigens in controls did not differ significantly from that found in a large pool of healthy subjects: 5/19 carried DR3 (26·3%) and there was one apparent DR3 homozygote. Only DR3 antigen was associated with variations in response to TT. The majority of controls carrying DR3 showed responses below the median (Table 2), an observation most striking for IgG-2 where the five DR3 subjects had a submedian response ($P=0{\cdot}016$, Fisher). No DR3 control had a total or subclass response above the highest MN responder.

The MN study group were recruited from a pool of MN patients whose immunogenetics have been fully analysed (Klouda *et al.*, 1979; Short, Dyer & Cairns, 1983). Eighteen of 20 were tested for DR antigens and 16/18 (88·8%) carried DR3 (including six apparent DR3 homozygotes). There was no evidence that the additional presence of another DR antigen was associated with a low response to TT. All six DR3 homozygotes had submedian responses for TT-IgG and all subclasses. The influence of DR3 on the responses is shown in Figs 1 and 2.

DISCUSSION

TT is an antigen encountered by most western children as part of routine immunization programmes, and is therefore a valuable tool for the study of secondary IgG immune responses in adults. The system had been well studied in recent years and there is evidence that the production of anti-TT IgG is promoted by antigen-specific T cells (Volkman, Allyn & Fauci, 1982; Friedman *et al.*, 1983) and may be suppressed subsequently by anti-idiotypic antibody (Geha, 1982; Geha, 1983).

There is some evidence that the response is age-dependent, being diminished in those over 65 years when compared with those under 35 (Kishimoto, Tomino & Mitsuya, 1980) although this

effect is less apparent when comparing 40–60 year olds with those over 60 (Solomonova & Vizev, 1973). In the present study no age influence was apparent in MN or controls.

Published data on subclass restriction of the TT response is conflicting. The predominance of IgG1 had been reported (Yount *et al.*, 1968); other methods report responses in all four subclasses (Parish, 1975; van der Giessen & Groeneboer-Kempers, 1976); while a small study with a sensitive RIA found IgG-3 and IgG-4 only (Shakib & Stanworth, 1980). In the present study responses to TT in all four subclasses were found by ELISA. Quantification of the responses was in arbitrary units based on comparison with the response in pooled human anti-TT immnoglobulins; however, for each subclass identical assays employing monoclonal antisubclass antibodies of comparable reactivity were used, and responses were detectable in the same range of dilutions, suggesting that absolute responses in the different subclasses were of the same order of magnitude. Comparison in ranks showed that the great majority had parallel responses in all four subclasses. There were a minority of exceptions in which response in single subclass was exaggerated or impaired; but such uneven responders were equally distributed between MN and controls and represented no definable group. One possible explanation for the inconsistency of published subclass data might be the genetic influence of Gm allotypes, which are known to influence IgG subclass levels (Litwin & Balaban, 1972; Yount, Kunkel & Litwin, 1967). Variation in TT response had been linked to the allotype G1m3 (Schanfield, 1980), but it would be difficult to test this possibility since greater than 90% of subjects in the UK (including those with MN) carry that allotype (Brenchley *et al.*, 1983).

Carney *et al.* (1980) studied the response to TT in subjects with different glomerular diseases. They suggested that the TT response (as judged by haemagglutinating antibody titres) was impaired, but their heterogenous study group contained only seven patients with MN on whom separate data was not given. The response was also impaired in a study of ten subjects with SLE (Nies *et al.*, 1980). Four out of ten subjects had lupus nephritis but details or renal histology were not reported, and some of those studied were receiving corticosteroids.

In the present study the TT response of those with MN was impaired when compared with controls for both total and IgG subclasses (although statistical significance was only reached in the case of IgG-3). Those with MN also had lower pre-immunization IgG-TT; after correction of post-immunization IgG-TT (by subtraction), levels in the MN group remained lower. However when only responders are considered, the post IgG-TT response is similar in MN and controls. Pre-immunization IgG-TT was poorly correlated with serum total IgG or degrees of proteinuria, and may simply reflect a greater interval since the previous TT immunization. This could not be determined since only a minority of those studied had exact documentation of the date of their last exposure to TT. It is not clear why IgG-3 should be most impaired. Although IgG-3 is a cationic subclass, the reduction in IgG-3-TT in MN did not have a profound effect on the total amount of cationic IgG-TT, since a predominance of cationic bonds was seen by autoradiography in MN (Fig. 3) as well as in controls.

Variations in serum IgG subclass levels do not appear to be important in diminishing the IgG-3 response, since in proteinuric patients with MN IgG-3 and IgG-4 are proportionally less depleted from serum than IgG-1 and IgG-2 (P. E. C. Brenchley, unpublished observations).

The significance of the low response in MN is uncertain. It is unlikely that it is secondary to the consequences of MN, since variations in IgG-TT did not associate with proteinura, renal impairment, hypoalbuminaemia or hypoglobulinaemia.

Recent experimental work (Oite *et al.*, 1982) suggests that electrical charge may be important in determining glomerular deposition of IgG, cationicity favouring subepithelial deposition. If such a mechanism were important in man, the IgG response in MN to a common antigen might be predominantly cationic, predisposing to subepithelial glomerular deposition of such IgG. However there was no increase in cationic IgG following immunization with TT in MN as compared to controls, nor was there striking restriction of the response to IgG-1 and IgG-3 (the most cationic subclasses). Intense cationic oligoclonal IgG bands have been reported recently in MN with heavy proteinuria and declining renal function (Coupes *et al.*, 1984); such bands were clearly seen on straightforward IEF with immunofixation. By contrast the bands seen in the TT response are multiple and of low intensity, being visualized only by autoradiography. It may be that TT is not a nephritogenic antigen in MN and that other common antigens will be found which do stimulate a restricted cationic IgG response.

The association of MN with HLA-DR3 is well recognised (Klouda *et al.*, 1979; Garavoy, 1980) and DR3 is also linked with a range of 'auto-immune' disorders (Klouda & Bradley, 1983). Apparently healthy subjects bearing DR3 may exhibit a range of immune abnormalities, including impaired reticuloendothelial Fc receptor function (Lawley *et al.*, 1981), enhanced spontaneous lymphocyte blastogenesis *in vitro* (Ambinder *et al.*, 1982) and impaired mitogen responsiveness (McCombs & Michalski, 1982). There is other evidence that B8-DR3 marks immune hyper-responsiveness, since B8-positive subjects with chronic active hepatitis are reported to have increased titres of autoantibodies and antibodies to common viruses when compared to their fellow sufferers who do not carry B8 (Galbraith, Eddleston & Williams, 1976). In this context it is not surprising that the TT response should show HLA restriction. The previous report (in a Japanese population) suggested an association of poor TT response with the haplotype A9-B5-DHO (Sasazuki, Kohno & Iwamoto, 1978). It is unusual that the present study associates DR3 with poor response, since hyper-responsiveness characterizes most of the other immunological features linked to DR3.

In summary, the IgG-TT response in MN is impaired as compared with controls, a phenomenon most marked for IgG-3. These changes occur independently of marked shifts in subclass or IgG spectrotype of the response. They appear to be associated with MN itself rather than the metabolic consequences of nephrotic syndrome, and may represent a further marker of the subtle immune defects which are associated with DR3.

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REFERENCES

AMBINDER, J.M., CHIORAZZI, N., GIBOFSKY, A., FOTINO, M. & KUNKEL, H.G. (1982) Special characteristics of cellular immune function in normal individuals of HLA-DR3 type. *Clin. Immunol. Immunopathol.* **23,** 269.

BANNISTER, K.M., HOWARTH, G.S., CLARKSON, Z.R. & WOODROFFE, A.J. (1983) Glomerular IgG subclass distribution in human glomerulonephritis. *Clin. Nephrol.* **19,** 161.

BATSFORD, S.R., TAKAMIYA, H. & VOGT, A. (1980) A model of *in situ* immune complex glomerulonephritis in the rat, employing cationised ferritin. *Clin. Nephrol.* **14,** 211.

BRENCHLEY, P.E.C., FEEHALLY, J., DORE, P., COUPES, B.M., SHORT, C.D. & MALLICK, N.P. (1983) Gm allotypes in membranous nephropathy. *New Engl. J. Med.* **309,** 556.

CARNEY, G., WALLS, R.S., GEORGE, C.R.P., PAYNE, J.E., NEWLAND, R.C. & LAWRENCE, J.R. (1980) Immunological function in glomerulonephritis. *Aust. NZ. J. Med.* **10,** 502.

COUPES, B.M., BRENCHLEY, P.E.C., FEEHALLY, J., SHORT, C.D. & MALLICK, N.P. (1984) Fluctuating cationic serum IgG in membranous nephropathy with declining renal function. *Abstr. 7th Int. Congress of Nephrology* 274A.

COUSER, W.G. & SALANT, D.J. (1980) *In situ* immune complex formation and glomerular injury. *Kidney Int.* **17,** 1.

DANILOVS, J.A., AYOUB, G. & TERASAKI, P.I. (1980) B lymphocyte isolation by thrombin-nylon wool. In: *Histocompatibility Testing* (ed by P.I. Terasaki) p. 287, UCLA Press, Los Angeles, USA.

FREIDMAN, S.M., PRINCIPATO, M.A., THOMPSON, G.S. & TEICHMAN, F. (1983) Antigen-specific and polyclonal immunoglobulin production induced by a cloned tetanus-specific T-cell line. *J. Immunol.* **130,** 1164.

GALBRAITH, R.M., EDDLESTON, A.W.L.F. & WILLIAMS, R. (1976) Enhanced antibody responses in active chronic hepatitis: relation to HLA-B8 and HLA-B12 and porto-systemic shunting. *Lancet*, **i,** 930.

GARAVOY, M.R. (1980) Idiopathic membranous glomerulonephritis and HLA associated disease. In: *Histocompatibility Testing* (ed. by P.I. Terasaki) p. 673, Williams and Wilkins, Baltimore, USA.

GEHA, R.S. (1982) Presence of auto-anti-idiotypic antibody during the normal human immune response to tetanus toxoid antigen. *J. Immunol.* **129,** 139.

GEHA, R.S. (1983) Presence of circulating anti-idiotype bearing cells after booster immunisation with tetanus toxoid and inhibition of anti-tetanus antibody synthesis by auto-anti-idiotype antibody. *J. Immunol.* **130,** 1634.

VAN DER GIESSEN, M. & GROENEBOER-KEMPERS, O. (1976) The subclass of human IgG antibodies against tetanus toxoid. *Clin. exp. Immunol.* **25,** 117.

HUNTER, W.M. & GREENWOOD, F.C. (1962) Preparation of iodine-131 labelled human growth hormone of high specification. *Nature*, **194,** 495.

KISHIMOTO, S., TOMINO, S. & MITSUYA, H. (1980) Age-related decline in the *in vitro* and *in vivo* synthesis of anti-tetanus toxoid antibody in humans. *J. Immunol.* **125,** 2347.

KLOUDA, P.T., MANOS, J. & ACHESON E.J. (1979) Strong association between idiopathic membranous glomerulonephritis and HLA-DRw3. *Lancet*, **ii,** 770.

KLOUDA, P.T. & BRADLEY, B.A. (1983) The interface between HLA genes and immunological disease. In: *Recent Advances in Clinical Immunology* 3 (ed. by R.A. Thompson & N.R. Rose) p. 92, Butterwoth, Guildford, UK.

LAWLEY, T.J., HALL, R.P., FAUCI, A.S., KATZ, S.I., HAMBURGER, M.I. & FRANK, M.M. (1981) Defective Fc-receptor functions associated with the HLA-B8/DRw3 haplotype. *New Engl. J. Med.* **304,** 185.

LITWIN, S.D. & BALABAN, S. (1972) A quantitative method for determining human γG allotype antigens (Gm). II: Differences in Gm expression for γG1 and γG3n sera. *J. Immunol.* **108,** 991.

MCCOMBS, C.C. & MICHALSKI, J.P. (1982) Lymphocyte abnormality associated with HLA-B8 in healthy young adults. *J. exp. Med.* **156,** 936.

NIES, K., TOYER, R., STEVENS, R. & LOUIE, J. (1980) Anti-tetanus toxoid antibody synthesis after booster immunisation in systemic lupus erythematosus. *Arth. Rheum.* **23,** 1343.

OITE, T. BATSFORD, S.R., MIHATSCH, M.J., TAKAMIYA, H. & VOGT, A. (1982) Quantitative studies of *in situ* immune complex glomerulonephritis in the rat induced by planted cationised antigen. *J. exp. Med.* **155,** 460.

PARISH, W.E. (1975) Some biological activities of IgG subclass antibodies after inoculation and in disease. In: *Allergy* (ed. by M.A. Ganderton, A.W. Frankland) p. 135, Pitman, London, UK.

SASAZUKI, T., KOHNO, Y. & IWAMOTO, I. (1978) Association between an HLA haplotype and low responsiveness to tetanus toxoid in man. *Nature,* **272,** 359.

SCHANFIELD, M.S. (1976) Genetic markers of human immunoglobulins. In: *Basic and Clinical Immunology* (ed. by H.H. Fridenberg, D.P. Stiles, J.I. Caldwell & J.V. Wells) p. 52, Lange, California, USA.

SHAKIB, F. & STANWORTH, D.R. (1980) Human IgG subclasses in health and disease. *La Ricerca Clin. Lab.* **10,** 463.

SHORT, C.D., DYER, P.A. & CAIRNS, S.A. (1983) A major histocompatibility system haplotype associated with poor prognosis in idiopathic membranous glomerulonephritis. *Disease Markers,* **1,** 189.

SOLOMONOVA, K. & VIZEV, S. (1973) Immunological reactivity of senescent and old people actively immunised with tetanus toxoid. *Z. Immunitaetsforsch* **146,** 81.

VOLKMAN, D.J., ALLYN, S.P. & FAUCI, A.S. (1982) Antigen-induced *in vitro* antibody production in humans: tetanus toxoid-specific antibody synthesis. *J. Immunol.* **129,** 107.

WALLS, R.S., GODFREY, P., NEWLAND, R.C., CARNEY, G. & LAWRENCE, J.R. (1982) Affinity of tetanus toxoid antibody in glomerulonephritis. *Clin. Immunol. Immunopathol.* **241,** 409.

WILLOUGHBY, E.W. & LAMBERT, A. (1983) A sensitive silver stain for proteins in agarose gels. *Ann. Biochem.* **130,** 352.

YOUNT, W.J., KUNKEL, H.G. & LITWIN, S.D. (1967) Studies of the Vi (gamma 2c) subgroup of gamma globulin. A relationship between concentration and genetic type among normal individuals. *J. exp. Med.* **125,** 177.

YOUNT, W.J., CORNER, M.M., KUNKEL, H.G. & KABAT, E.A. (1968) Studies on human antibodies. Vi-selective variations in subgroup composition and genetic markers. *J. exp. Med.* **127,** 633.