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IgG subclass distribution in organ specific autoantibodies. The relationship to complement fixing ability

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SUMMARY

Indirect immunofluorescence techniques employing sheep monospecific antisera to human IgG subclasses on unfixed cryostat sections have revealed the IgG subclass distribution in autoantibodies to pancreatic islets (ICA), thyroid epithelial (TMA), gastric parietal (PCA) and adrenal fasciculata (AdA) cells. Whereas antibodies were detected in all four subclasses in 21 of 27 TMA positive sera (mean IgG titre $29 \pm 5{\cdot}2$), 13 of 15 PCA (mean IgG titre $41 \pm 3{\cdot}8$) and eight of 14 AdA sera (mean IgG titre $10 \pm 3{\cdot}1$), only four of 35 ICA positive sera (mean IgG titre $45 \pm 3{\cdot}6$) reacted in all four subclasses. Approximately 50% of ICA positive sera showed a restricted polyclonal response to the 'common' pancreatic antigen and 12% of these sera reacted only with IgG2 subclass. The restriction rarely applied to co-existent thyrogastric antibodies in these sera and was independent of the ability of ICA to fix complement. Lesser subclass restrictions were observed in antibody responses to the 'common' antigen of the adrenal cortex.

INTRODUCTION

It has been demonstrated that islet cell antibodies (ICA) are invariably of IgG class when tested by indirect immunofluorescence (IFL) on frozen sections of human pancreas. By the same method approximately 55% of ICA IgG fix complement (Bottazzo *et al.*, 1980). CF-ICA are now established as better markers than ICA IgG for the onset of clinical diabetes in genetically predisposed individuals (Gorsuch *et al.*, 1981).

There is increasing evidence that selectivity of particular IgG subclasses may be associated with antibody–complement interactions. In some systems IgG3 has a greater capacity to fix complement than other IgG subclasses and this ability decreases in the order IgG3 > IgG1 > IgG2 > IgG4 (Shakib & Stanworth, 1980). Restricted subclass responses have been demonstrated in some autoimmune conditions. For instance, in two cases of myasthenia gravis the acetylcholine receptor antibodies were shown to be exclusively IgG3 (Lefvert & Bergstrom, 1977; Fulpius, Miskin & Reich, 1980). CF antibodies to epidermal basement membrane in *Herpes gestationis* were mainly IgG1 (Carruthers & Ewing, 1978) and in bullous pemphigoid, CF antibodies were found in IgG subclasses 1, 3 & 4, whereas non-CF sera contained IgG4 antibodies only (Sams & Schur, 1973). Anaemia in Gambian children with falciparum malaria is associated with IgG1 red cell antibodies, whereas non-CF IgG2 or IgG4 antibodies do not cause RBC destruction (Facer, 1980).

Recognized methods for the study of subclasses such as coprecipitation with radiolabelled

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antigen (Farr, 1958; Torrigiani, Roitt & Doniach, 1968) immunoelectrophoresis (Terry & Fahey, 1964) or radial immunodiffusion (Yount, Kunkel & Litwin, 1967; Schur *et al.*, 1970) were not applicable to this study since the pancreatic antigen is not fully characterized (Baekkeskov *et al.*, 1982). We therefore decided to examine the subclass distribution of ICA and other organ specific antibodies using the conventional indirect IFL technique on cryostat sections of the corresponding human tissue.

MATERIALS AND METHODS

Patient sera. A total of 91 sera selected from well documented autoimmune polyendocrine patients and relatives participating in the Barts–Windsor prospective family study of type I DM (Gorsuch *et al.*, 1980) were studied. All sera were stored at $-20°C$ for a period of < 2 years without heat-inactivation.

Substrates. Cryostat sections (4μm) of blood group O human pancreas, thyrotoxic thyroid, stomach and adrenal were employed. Pancreas and adrenal sections were fixed in acetone for 1–3 min prior to use. To minimise discrepancies due to variation in antigen strength, all sera were tested on serial sections cut from blocks of individual tissues of pre-tested antigenic potency.

Antisera. (a) Polyclonal sheep antisera to human IgG subclasses were obtained from the Immuno Diagnostic Research Lab., Medical School, Birmingham, UK (batch Nos: αIgG1, Z442H; αIgG2, Z524R; αIgG3, Z427D; α IgG4, Z527K), from Seward Labs, London (batch Nos: α IgG1, Z843J & BX503; α IgG2, Z868H, BX04 & 1366; α IgG3, Z783D, BX05 & BA37; α IgG4, Z756C, BX06 & BA38) and from Miles Labs, Elkhart, Indiana, USA (α IgG3, code 64–312). The specificity of these antisera was cross-checked on smears of myelomatous bone marrow aspirates and no cross-reactivity was found by IFL. The antisera were used at an optimal dilution of 1/10 and not more than one titre difference was detectable when successive batches of the same anti-subclass specificity were compared using sera of high and low autoantibody titre. α IgG3 (Miles) was likewise found to give results < 1 titre difference from those obtained using α IgG3 (Seward). Rabbit anti-sheep FITC conjugate (Dako Labs. Denmark) was adsorbed at 1/10 dilution with human liver powder prior to use.

(b) Mouse ascites fluids containing monoclonal antibodies to human IgG subclasses (α IgG1: BAM09; α IgG2: BAM10 & BAM14; α IgG3: BAM08; α IgG4: BAM11 & BAM13 and the clone supernatants corresponding to BAM 09,10 & 14) were kindly donated by Seward Labs. The monoclonal antibodies were applied at doubling dilutions (range 1–100) following incubation of tissue sections with undiluted sera of high IgG titre (range 200–400) for ICA and TMA. After washing, the sections were treated for 30 min with sheep, horse or rabbit anti-mouse FITC conjugates pre-adsorbed with human IgG and/or human liver powder.

All sera were tested at a minimum of four doubling dilutions for end point determination which was expressed as the inverse highest dilution at which the organ specific antibodies were detectable. The sections were viewed using a Zeiss microscope with epi-illumination (magnification 300–500). Titres for IgG and CF were obtained using sheep anti-human IgG and C3 FITC conjugates (Wellcome Labs) respectively (Bottazzo *et al.*, 1980).

RESULTS

The ascites fluids and clone supernatants containing monoclonal anti-human IgG subclasses failed to detect organ specific antibodies under the conditions described above. Hence, the observations reported here relate to antibody titres obtained using polyclonal IgG monospecific antisera. The range of mean subclass titres in TMA (see Table 2) confirmed that the polyclonal antisera to all four subclasses were highly reactive employing IFL techniques.

The majority of sera with autoantibodies to thyroid microsomes, gastric parietal cells and adrenal cortex reacted in all four IgG subclasses (Table 1). In these tissues only low titre sera lacked either IgG3 or both IgG3 and IgG4. For ICA, however, despite a higher mean IgG titre than that of

Table 1. IgG subclass detection in human organ specific autoantibodies

Antibody	No. of sera studied	IgG subclasses 1+2+3+4	1+2+4	1+2	2+4	2 alone
ICA	35	4 80±3·1 (20–200)	14 70±2·6 (20–800)	10 41±3·0 (4–200)	3 22±3·7 (10–100)	4 6±1·6 (4–10)
TMA	27	21 45±5·6 (2–800)	4 7±2·7 (2–20)	2 6±5·1 (2–20)	0	0
PCA	15	13 43±4·2 (2–200)	1 (20)	1 (20)	0	0
AdA	14	8 12±2·2 (4–20)	3 22±4·5 (5–100)	3 3±1·7 (2–5)	0	0

The relevant number of sera is shown together with the corresponding IgG titre (geometric mean ±s.d.) and the titre range in parentheses below.

TMA and PCA and six-fold higher than the mean AdA titre (Table 2), subclass 3 was detected in only four (12%) of the 35 sera investigated and 17 (49%) of these sera were restricted to either subclass 2 alone or subclasses 2+1 or 2+4.

Table 2 indicates that the cytoplasmic staining reactions for TMA and PCA were similar for all four subclasses, but in ICA the mean IgG4 titre was considerably lower than that of either IgG1 or IgG2. In AdA, IgG4 titres were comparable with those of subclasses 1 and 2, the mean IgG3 titre being lower than that of the other subclasses.

In TMA a good correlation between IgG and ICFT titres was observed ($P < 0{\cdot}001$ using Spearman's ρ test). By contrast, CF-ICA titres did not correlate with individual ICA IgG titres. In the absence of IgG3, detection of IgG1 did not necessarily concur with CF and ICA of the same IgG titre (200) containing all four subclasses could display a 10-fold difference in ICFT titre (20:200).

Specific examples of sera displaying multiple autoantibodies (Table 3) indicate with one exception (case No. 7) that subclass restriction in ICA is not accompanied by parallel restrictions in coexistent antibodies, irrespective of titre. There is some evidence that restrictions in AdA occur

Table 2. Comparative IgG, IgG subclass and complement fixation (ICFT) titres in cytoplasmic autoantibodies to pancreatic islet (ICA), thyroid epithelial (TMA), gastric parietal (PCA) and adrenal fasciculata cells (AdA) of human origin

Antibody	IgG	IgG1	IgG2	IgG3	IgG4	ICFT
ICA	45±3·6 (4–800) $n=35$	22±4·5 (1–400) $n=28$	16±3·8 (2–200) $n=35$	2±1·4 (1–2) $n=4$	5±3·3 (1–50) $n=21$	16±3·8 (1–200) $n=26$
TMA	29±5·2 (2–400) $n=27$	18±4·4 (2–400) $n=27$	23±4·5 (2–800) $n=27$	18±3·5 (2–200) $n=21$	17±4·7 (1–800) $n=25$	22±4·8 (2–800) $n=23$
PCA	41±3·8 (2–200) $n=15$	20±2·7 (5–100) $n=15$	19±3·0 (2–100) $n=15$	9±2·3 (2–20) $n=13$	13±2·4 (2–50) $n=14$	40±3·6 (5–200) $n=15$
AdA	10±3·1 (2–100) $n=14$	7±2·5 (1–20) $n=14$	7±2·8 (1–20) $n=14$	2±1·9 (1–5) $n=8$	6±2·7 (1–20) $n=11$	7±2·5 (2–20) $n=8$

The geometric mean titre value is shown, together with the range for positive observations (n).

Table 3. Specific examples of IgG subclass and complement fixation (ICFT) titres in sera displaying multiple autoantibodies.

Case No.	Sex/ age	Diagnosis/ duration (Yr)	Antibody	IgG1	IgG2	IgG3	IgG4	ICFT
1	M/50	Polyendocrine,	ICA	2	2	—	—	1
		IDDM, vitiligo	TMA	2	2	2	2	—
		PA	PCA	10	10	10	10	100*
2	F/47	Mother of diabetic,	ICA	—	5	—	—	5
		thyrotoxic	TMA	50	50	20	20	20
3	F/53	IDDM (15)	ICA	—	10	—	—	10
		Myxoedema	TMA	10	20	10	10	5
			PCA	20	50	20	50	50
4	F/63	IDDM,	ICA	—	20	—	—	10
		myxoedema	TMA	10	20	10	10	10
			PCA	50	50	20	50	200*
5	F/63	Polyendocrine,	ICA	200	200	1	20	200
		DM(2), PA,	TMA	20	20	10	20	10
		vitiligo	PCA	20	20	5	10	100*
6	F/	IDDM (pregnant)	ICA	400	200	—	50	200
			TMA	100	50	20	50	50
7	M/43	Addison's (5)	AdA	5	5	—	—	—
			TMA	5	10	—	5	5
			PCA	20	20	—	10	20
8	M/35	Addison's (6)	AdA	5	5	—	1	—
			TMA	2	5	—	—	—
			PCA	5	10	—	—	20
9	F/	Polyendocrine,	AdA	20	20	—	20	10
		DM, myxoedema	TMA	20	20	—	5	20
			PCA	50	50	20	20	100
			ICA	20	10	—	5	20

DM = diabetes mellitus; IDDM = insulin-dependent diabetes mellitus; PA = pernicious anaemia. None of these sera contained ICA of IgM or IgA class.

* High ICFT titres presumed due to the presence of IgM detected in these sera for PCA.

concurrently with those of other antibodies (case Nos 7–9), but further studies are needed to clarify this observation.

DISCUSSION

The mean subclass titres for the four autoantibodies studied (Table 2) indicate that the relative affinities of the γ-globulin subclasses for tissue antigens do not correlate with their distribution in normal human sera, which is 64–70% of total for IgGl, 23–28% IgG2; 4–7% IgG3 and 3–4% IgG4 (Schur, 1972). A previous report of the relative antigen binding capacity for IgG subclasses in autoantibodies (Hay & Torrigiani, 1973), found the mean percentage binding to be within the above range for anti-thyroglobulin antibodies in sera from patients with Hashimoto's disease. In our study, although similar polyclonal responses were observed in all four subclasses for thyroid and gastric antibodies and in three subclasses (1, 2 & 4) for adrenal antibodies, the response in islet cell antibodies was generally restricted for subclasses 1 & 2 and markedly reduced in subclass 3.

IgG3 have a high tendency to aggregate and antibodies of this class are frequently found in patients with cryoglobulins (Grey *et al.*, 1968). However, attempts to release bound or aggregated

antibodies from ICA positive sera (known to contain high molecular weight, Clq binding complexes) failed to release either IgG3 antibodies or to raise the ICA titres (Bodansky *et al.*, 1982).

The clonality of thyroglobulin antibodies investigated by isoelectric focussing has revealed a restricted polyclonal response in only 1/31 sera, though there was evidence of clonal dominance in some (Nye, Carvalho & Roitt, 1979). The response of several species to a number of carbohydrate antigens is subclass restricted, e.g. human antibodies to dextrans and levans, techoic acid and streptococcus Group A polysaccharide have been detected only in subclass 2 (Yount *et al.*, 1968; Perlmutter *et al.*, 1978). Whether the differing subclass responses in polyendocrine cases displaying multiple autoantibodies (Table 3) are attributable primarily to the carbohydrate nature of the autoantigenic epitopes and whether the response is controlled by genes in the HLA region or in other chromosomes (Gm) is unknown.

In newly diagnosed type I diabetics the CF-ICA represent approximately 50% of the total prevalence of ICA IgG; the titre is usually lower and they tend to disappear more rapidly from the circulation than ICA IgG. The inference here is that in any one individual, CF antibodies represent a fraction of the total ICA response. It is evident from the diversity of CF-ICA titres with respect to IgG subclasses, that this fraction may vary markedly amongst individuals. In systemic lupus erythematosus titres of CF antibodies to ds DNA are unrelated to the absolute quantity of anti-ds DNA present (Beaulieu *et al.*, 1979). The presence of CF antibodies can be related to the lesions in this disease (Tojo, Friou & Spiegelberg, 1970; Beaulieu, Quismorio & Friou, 1977; Chubick *et al.*, 1978) and although anti-DNA antibodies have been detected in a number of sera in all four subclasses, (Schur, Monroe & Rothfield, 1972), there is a predominance of IgG1 and IgG3 in patients with severe nephritis (Puritz *et al.*, 1973; Sontheimer & Gilliam, 1978).

We have shown that CF-ICA relate more closely to the clinical onset of diabetes than non-complement fixing ICA and have postulated that the CF antibodies reflect damage of pancreatic beta cells more selectively than ICA IgG (Bottazzo *et al.*, 1981), probably because the CF moiety contains beta cell specific cytotoxic antibodies (Van de Winkel *et al.*, 1982). Our results show no preponderance of either IgG3 or IgG1 amongst CF-ICA (Table 3). The low incidence of IgG3 in ICA may contribute largely to this apparent anomaly, but it could be attributed to the fact that our methods measure CF with the antigens *in situ*, in contrast to the customary assessment of CF by binding to artificially formed soluble antigen–antibody complexes. The Fc fragments of IgG1 and IgG4 have similar binding capacities for Clq (Isenman, Dorrington & Painter, 1975), which has led to the suggestion that shielding by the Fab regions of the molecule prevents IgG4 from fixing complement. Attachment of IgG antibodies to antigens *in situ* could effect allosteric changes that do not occur with soluble antigen–antibody complexes.

Although an acceptable explanation for the variable complement fixing capacity in ICA is that sera having only ICA IgG react with very few epitopes so that the IgG molecules are too far apart to fix complement, it is clear that amongst individual sera CF-ICA titres are not directly related to individual subclass titres. This observation suggests that variable region genes may well contribute to the conformational ability of an IgG subclass to expose reactive sites for complement binding. It has already been shown by Ey, Prowse & Jenkin (1979) that mouse IgG1 can exist as isotypes that may or may not fix complement and Stanislawski & Mitard (1976) have also demonstrated the existence of two antigenically distinct mouse IgG1 myeloma proteins.

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