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THE NATURE OF AUTOANTIGENS

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1. Autoimmunity in disease is driven by autoantigen; 2. Cell surface molecules may stimulate autoreactive T-helpers if class II MHC is expressed; special factors may predispose to the ease of class II induction; 3. Soluble autoantigens may be focussed by primed B-cells and processed for presentation to T-cells; 4. Autoantigenicity may be influenced by metabolic events: (a) Poorly iodinated thyroglobulin does not induce thyroiditis; (b) IgG rheumatoid arthritis has galactose deficient Fc oligosaccharides.

Glycosylation defects may prove to have wide implications.

It is generally accepted that most if not all autoantigens associated with autoimmune disorders are freely accessible to circulating lymphocytes. Since there is considerable evidence that autoreactive t- and B-cells are present in normal individuals, a variety of mechanisms operate to prevent their triggering. Hitherto, most studies have looked for defects in immune responsiveness to account for the development of autoimmunity, but these diseases appear to have a multifactorial basis and present communication discusses possible abnormalities in the autoantigens themselves and their presentation.

ABERRANT EXPRESSION OF CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX (HMC) MOLECULES

Potentially autoantigenic surface molecules cannot interact with their corresponding autoreactive T-helper in the absence of class II MHC. The important observation that thyroid cells in Graves' disease were inappropriately expressing class II molecules showed that this defence against autoimmunity can be breached when genes coding for class II are derepressed (Bottazzo et al., 1983). It was also realized that in the human pancreatic islets of Langerhans, the insulin-producing β-cells which are the target for attack, express class II molecules on their surface in contrast with the somatostatin and glucagon-producing cells which are negative for class II (Bottazzo et al., 1985) (Table). Interestingly, whereas γ-interferon (IFN γ) switches on class II expression in normal human thyroid cells *in vitro*, it does not readily do so with normal pancreatic islet cells, although it is active if given together with tumour necrosis factor (Pujol-Borrell et al., 1987). However, in these experiments, somatostatin and glucagon cells also became positive for class II and in these circumstances it is intriguing to note that one of the present authors (A. Cooke) and her colleagues (Walker et al., 1986) have shown that IFN γ alone can *selectively* switch on class II in insulin-producing cells in BB-rats and NOD mice at a time when they are prone to develop autoimmune diabetes spontaneously (Table). This strongly suggests that some other factor may predispose the β-cells to express class II in response to agents like IFN γ; the retrovirus which integrates with the β-cells of the NOD mouse pancreas may be one such agent.

FOCUSSED AUTOANTIGEN PRESENTATION BY PRIMED B-CELLS

It is only comparatively recently that convincing evidence has emerged for the involvement of B-cells in antigen presentation (e.g. Lanzavecchia, 1985). Because of the unique binding property of the Ig receptor, the antigen concentration for effective presentation may be many times lower than that needed by a non-specific antigen presenting cell (APC) (Abbas et al., 1985). In autoimmunity where antigen concentration may be limiting, presentation by B-cells could play an important part in the development of disease, particularly as it has been shown that such autoantigen-specific B-cells do exist in the repertoire of normal individuals. To test this hypothesis, in collaboration with P. Hutchings and D. Rayner (Hutchings et al., 1987) we have employed as a source of APC, normal murine B-cells which have been primed *in vivo* to the autoantigen thyroglobulin (Tg). B-cells obtained in this way were tested for their efficiency at presenting Tg to CH9, an Lyl^+2^-, $L3T4^+$, $I\text{-}A^k$ restricted T-cell hybridoma specific for Tg (Rayner et al., 1987).

When Tg primed spleen cells were cultured with CH9, a concentration of 0.1 to 1.0 μg/ml Tg was sufficient to activate the hybridoma

TABLE

Stimulation of Class II MHC on pancreatic islet cells

Islet cells	Stimulant	Expression of Class II on:		
		Insulin cells	Somatostatin cells	Glucagon cells
* Diabetic human	None	++	–	–
Normal human	IFNγ	–	–	–
Prediabetic BB rat	IFNγ	++	–	–
Diabetic resistant rat	IFNγ	–	–	–
Normal human	IFNγ + TNF	++	++	++

* In vivo

cells. Activation was measured by increased production of IL-2 which was assayed using an IL-2-dependent CTL line as an indicator cell. In contrast, when non-primed spleen cells were used as APC, comparable activation was only seen at an antigen concentration of 50μg/ml. High efficiency antigen presentation by primed cells at 1μg/ml was unaffected by the removal of T-cells (anti-Thy 1.2 treatment), whereas B-cell removal (treatment with the B-cell specific monoclonal antibody LR-1; a gift from S. Marshall-Clarke, University of Liverpool) completely abrogated presentation at 1μg/ml while leaving presentation at 50μg/ml intact (Fig. 1). This indicates the presence of at least two distinct antigen presenting populations in primed spleen cells which vary both in their sensitivity to LR-1 treatment and in the range of antigen concentrations over which they are active, presumably highly efficient B-cells and less efficient macrophages or dendritic cells. In non-primed spleen cells, only the low efficiency component was detectable (at 50μg/ml) and this was resistant to both anti-Thy 1.2 and LR-1 treatment.

We have shown also that not only do Tg-primed B-cells present antigen to CH9 at low concentrations, but they can subsequently be induced by the stimulated CH9 to secrete anti-Tg antibodies. Although it is unlikely that there are enough antigen specific B-cells in non-primed animals to contribute significantly to presentation of antigen during a primary response. B-cell presentation may represent a means by which an initial triggering event, priming both B- and T-cells, may allow maintenance of autoreactive responses *in vivo* in the presence of low concentrations of circulating antigen.

AUTOANTIGENIC VARIATION

The role of iodination in autoantigenicity of thyroglobulin — The restrictions on normal T-cell help for autoreactive B-cells may be bypassed if the autoantigen becomes altered in such a way as to present new carrier determinants which can be recognized by non-tolerized T-helpers. A familiar example of this is the induction of autoantibodies by cross-reacting antigens. The following section which represents work carried out in collaboration with B. Champion, D.C. Rayner, P. Byfield, K. Page and J. Chan, examines possible variation in the autoantigenicity of thyroglobulin in relationship to the degree of iodination.

A number of studies have suggested a link between the level of dietary iodine and the incidence of thyroid autoimmunity both in man (Beierwaltes, 1969) and experimental animals (Bagchi et al., 1985). Since the thyroid gland incorporates iodine into Tg for the synthesis of thyroid hormones thyroxine (T_4) and tri-iodothyronine (T_3), it seems reasonable to suggest that iodination influences the immunogenicity of Tg. Sundick and colleagues (Sundick et al., 1987) have shown recently that highly iodinated chicken Tg is more autoimmunogenic than poorly iodinated Tg. Althought the immunologic basis for this observation is not yet clear, one possibility is that T-cells will only recognise Tg when it is sufficiently iodinated. We have analyzed a mouse autoreactive T-cell clone (MTg9B3) specific for murine Tg for its ability to recognize Tg iodinated to different degress. A second, independently-derived cloned T-cell population gave similar results.

The mouse autoreactive Tg-specific clones have been shown to recognize an epitope which is also present on human Tg (Rayner et al.,

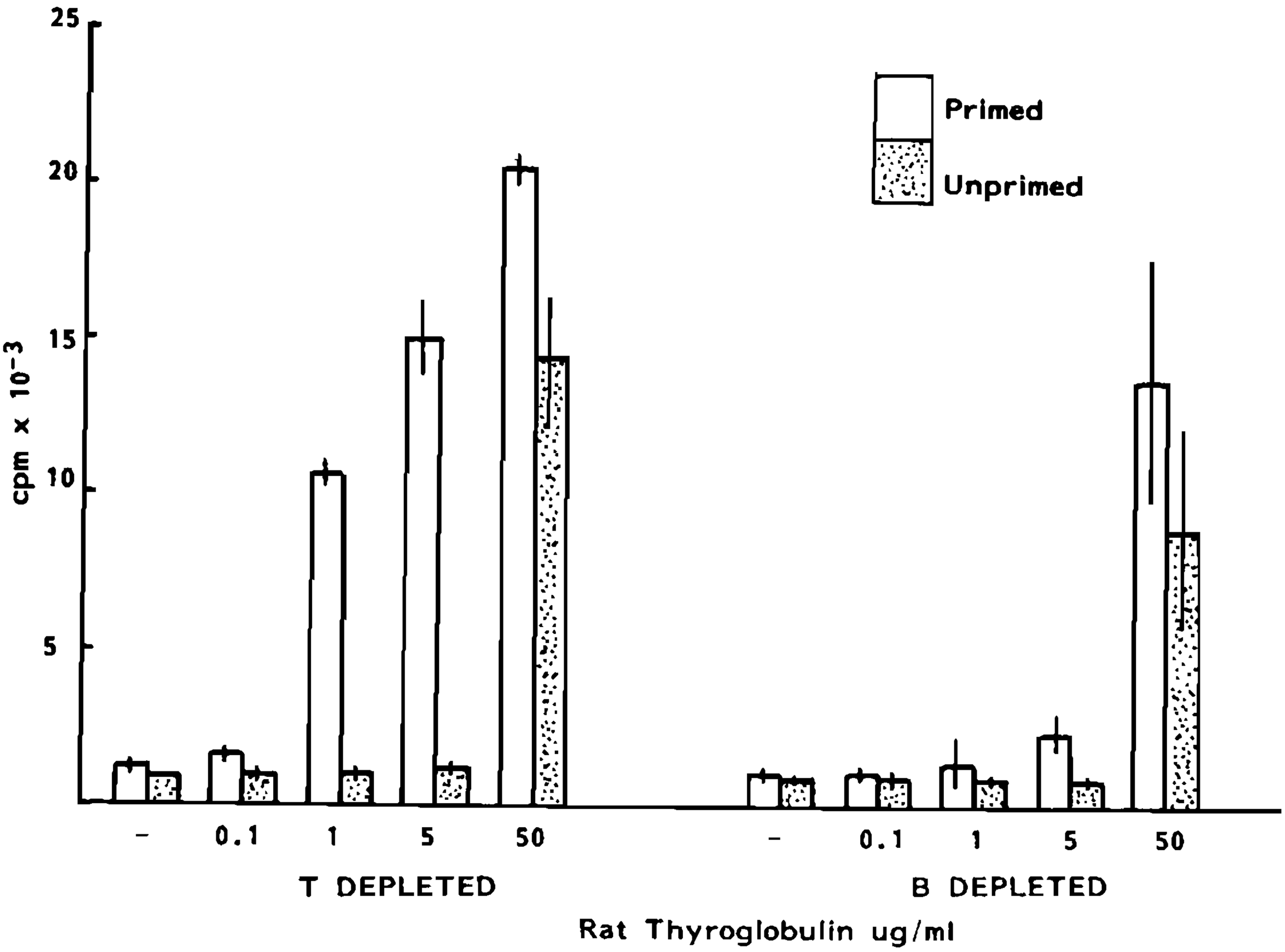

Fig. 1: Effect of T- or B-lymphocyte depletion on presentation of thyroglobulin to the T-cell hybridoma CH9 by thyroglobulin-primed murine spleen cells. Results are expressed in terms of IL-2 release measured by the effect on [^{125}I] uridine incorporation into an IL-2 dependent cell line.

1987; Champion et al., 1985). We tested MTg9 B3 for its ability to respond to a panel of human Tg's, which differed in their degree of iodination (as measured by their content of T_4 and T_3). As shown in Fig. 2, the level of response was dependent upon the degree of Tg iodination. Thyroglobulins with very low iodine content (JO.08 in Fig. 2 and others not shown) failed to stimulate the T- cells. The level of response did not show a strict rank-order correlation with iodine content, but this is not surprising since a number of tyrosine residues can be iodinated and it is not yet known which region of the Tg' molecule is recognized by the T-cell clone.

To substantiate these observations, we prepared normal (iodinated) and non-iodinated mouse Tg from matched groups of mice. Non-iodinated Tg was produced by supplementing the diet with the peroxidase-blocking drug, aminotriazole (ATA), for 3-6 weeks prior to collecting the thyroids. This procedure induced large goitrous thyroids, the Tg from which was shown to have undetectable levels of T_4 (compared with approximately 3 T_4 residues por mole for normal Tg). These Tg preparations were then examined for their ability to trigger MTg9B3. Two separate preparations of non-iodinated Tg (ATA Tg) were unable to stimulate the T-cell clone, whilst the normally-iodinated Tg preparations triggered the cells perfectly well. This confirms the observation made with human Tg preparations, that this T-cell clone will only recognize Tg if it is sufficiently iodinated. As stated previously, this conclusion is also true for another independently-derived Tg-specific T-cell clone.

This observation in the mouse appears to be specific for T-cells since, unike data from chickens (Sundick et al., 1987), Tg autoantibodies (both polyclonal and monoclonal) fail to discriminate between normal and non-iodinate autologous Tg preparations (data not shown). We have now found that poorly iodinated thyroglobulin is unable to induce thyroiditis in mice when injected with the appropriate adjuvant although it is only slightly less effective than the iodinated protein in provoking auto-

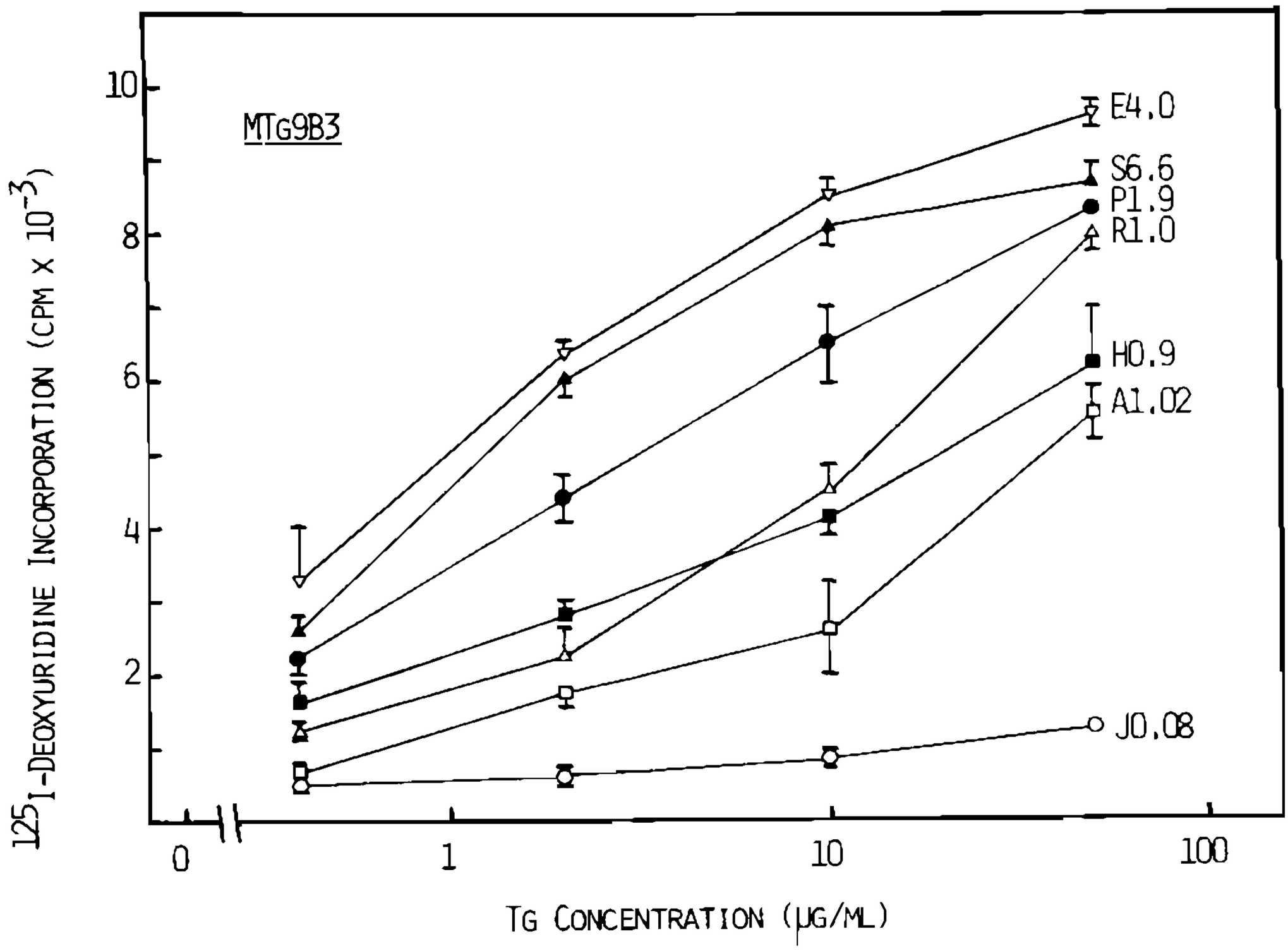

Fig. 2: Proliferative response of mousee T-cell clone raised against murine thyroglobulin of different iodine content. The degree of iodination of each different preparation is given by the thyroxine content in residues per mole of thyroglobulin. Very poorly iodinated material produced no proliferation.

antibody formation. This implies that iodination of Tg is a requirement for recognition by T-cells involved in pathogenesis but not for all T-helper cells important in antibody production.

The mechanisms through which tolerance to self Tg is maintained are incompletely understood, but circulating Tg appears to be poorly iodinated (Schneider et al., 1983), particularly in neonates (Etling, 1977), T-cells specific for certain iodination-dependent determinants may be relatively protected from clonal deletion or inactivation mechanisms. In certain susceptible individuals (or animal strains), access of more completely iodinated Tg to the peripheral tissues, which may occur following TSH stimulation (Schneider et al., 1985) or injection of Tg into experimental animals, could break tolerance and lead to autoimmunity. It is tempting to speculate that the influence of dietary iodine on human thyroid autoimmunity might be due to a similar mechanism to that described in the present paper.

Defective glycosylation of IgG in Rheumatoid arthritis – In view of the considerable body of evidence consistent with the view that autoreactivity to IgG may be a dominant factor underlying the chronic inflammatory changes leading to the production of an erosive pannus in rheumatoid arthritis (RA), it was of particular interest that a recent paper by Parekh et al. (1985) described an increase in galactose-free oligosaccharides in the IgG of patients with rheumatoid arthritis. The defect appears to be associated with the Fc rather than the Fab oligosaccharides. The two N-linked Fc oligosaccharides (one on each C_H2 domain), hold the two C_H2 domains apart through their $1 \rightarrow 3$ chains while the galactose on the $1 \rightarrow 6$ chain fits snugly into a cavity on the domain surface.

D. Isenberg, G. Rook and one of the authors (I.M. Roitt) have been collaborating with the Oxford group (R. Parekh, T. Rademacher and R. Dwek) to extend these studies and look at disease specificity. Investigations on further RA patients confirmed the original observations, although there was slightly more overlap with the normal population than was reported in the earlier paper due to an unexpected rise in the percentage of galactose-free chains with age

from about 30 years onwards. This glycosylation defect was not a general feature of rheumatological disorders since primary Sjogren's syndrome, 'primary' SLE, ankylosing spondylitis and psoriatic arthropathy were normal, although a proportion of patients with SLE plus Sjogren's were positive possibly due to overlap with features of RA. Of other chronic inflammatory conditions looked at as controls, Crohn's disease and surprisingly, tuberculosis showed the galactose defect; this was not a general feature of mycobacterial infection however, since leprosy patients were normal in this respect.

Preliminary studies by J. Axford suggest that the galactose transferase enzymes in B- but not T-cells are depressed. The presence of abnormal numbers of oligosaccharide chains lacking galactose, particularly if this occurs on both C_H2 domains in the same Fc could provide novel features which might well influence autoantigenicity. In addition, the cavity on the C_H2 vacated by the absent galactose could act as a binding site for the Fab sugars on self-associated IgG rheumatoid factors thereby enhancing their stability as immune complexes. These findings open up exciting new immunomodulatory approaches to RA and perhaps other diseases, and serve to emphasize the importance of potential abnormalities in autoantigens in the aetiology and pathogenesis of autoimmune disorders.

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