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A transient rise in agalactosyl IgG correlating with free interleukin 2 receptors, during episodes of erythema nodosum leprosum

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SUMMARY

The proportion of oligosaccharide chains on the Fc fragment of IgG which terminate with *N*-acetylglucosamine and not galactose (%GO) has previously been shown to be raised in rheumatoid arthritis (RA), Crohn's disease (CD) and tuberculosis (Tb), but to be normal in sarcoidosis (SA), and in both lepromatous and tuberculoid leprosy. However we have now studied %GO in sequential serum samples collected from lepromatous leprosy patients undergoing episodes of erythema nodosum leprosum (ENL). During ENL %GO is transiently raised, and this rise parallels an increase in circulating interleukin 2 receptors (IL-2R). These findings confirm that changes in T cell function occur during ENL. Moreover it appears that %GO rises when there is, simultaneously, T-cell-mediated tissue damage and an acute phase response (RA, CD, Tb, ENL), but not when there is an acute phase response without major T cell involvement, or chronic T cell activity alone (SA, and tuberculoid leprosy). We suggest therefore that %GO is an indicator of a type of T cell activity with broad immunopathological implications.

Keywords leprosy tuberculosis interleukin 2 receptor erythema nodosum leprosum agalactosyl IgG

INTRODUCTION

There is a conserved *N*-glycosylation site on the Fc of IgG, at Asn 297 on the CH2 domain. This site bears essentially non-sialylated (90%) biantennary oligosaccharides, a variable proportion of which bear terminal galactose on one or both of their outer arms. When galactose is not present the terminal sugar is usually *N*-acetylglucosamine (GlcNAc). In sera from normal donors the proportion of oligosaccharides bearing no terminal galactose (agalactosyl IgG or GO) decreases from a level of about 30% in small children to a trough of ~20% at the age of 25 years. Then it increases steadily with age to ~40% by 70 years of age (Parekh *et al.*, 1988). In rheumatoid arthritis, tuberculosis, and Crohn's disease there is an increase in GO which parallels the activity of the disease (Parekh *et al.*, 1985; Rademacher *et al.*, 1988). Diseases in which no changes in %GO are seen include systemic lupus erythematosus (SLE) (unless Sjögrens syndrome is also present), osteoarthritis, multiple sclerosis, sarcoidosis, *Klebsiella* infections, myositis, and a range of viral infections. Moreover, in contrast to the findings in tuberculosis, levels are not normally raised in either tuberculoid or lepromatous leprosy (Rademacher *et al.*, 1988).

However, we report here that there is a rise in %GO during episodes of erythema nodosum leprosum (ENL). We also confirm a previous report that there is a rise in serum levels of free interleukin 2 (IL-2) receptors during ENL (Tung *et al.*, 1987), and these two assays show some correlation.

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MATERIALS AND METHODS

Patients

Seven lepromatous leprosy patients undergoing episodes of ENL were studied at the Victoria Hospital, Dichpalli, Andhra Pradesh, India. All were clinically graded according to the Ridley–Jopling classifications (Ridley & Jopling, 1966), and skin smears were examined for morphological and bacteriological indices (MI and BI). They had received dapsone and either clofazimine or rifampicin for varying periods prior to the ENL episodes. ENL was treated with steroids, usually prednisolone, and/or thalidomide. Serum samples were taken at intervals, before (where possible), during and after the episodes, and detailed records were made of the activity of the ENL at the time of each bleed. Details of these patients are shown in Table 1.

Sera from tuberculosis patients of mixed age and race were samples acquired before, or within 1 month of starting conventional therapy. They were selected at random from the collection held at UCMSM.

Monoclonal antibody to terminal N-*acetylglucosamine (GlcNAc)*

The monoclonal antibody used for the immunoassay of agalactosyl IgG has been described elsewhere. Briefly, mice were immunized with Group A streptococcal cell wall peptidoglycan/polysaccharide complex, and monoclonals were selected for binding to asialo-agalacto-fetuin, and to agalactosyl IgG (Rook, Steele & Rademacher, 1988).

Standards for the immunassay for agalactosyl IgG

Serum and IgG samples, the GO values of which had been obtained by a previously published biochemical procedure, were used as standards throughout this study. Briefly, the *N*-linked oligosaccharides were released from the IgG, using anhydrous hydrazine, and purified. The percentage of these oligosaccharides bearing no terminal galactose residues (GO) was then determined by measuring the hydrodynamic volume after exposure to an exoglycosidase mixture (Parekh *et al.*, 1985). The set of standards used was derived from normal donors and tuberculosis patients.

Immunoassay for agalactosyl IgG

Using a template and sharp scalpel, nitrocellulose sheets were shaped into 'combs' with twelve teeth, spaced so that each tooth could enter a microtitre well, while the backbone of the comb was supported on the sides of the wells. The entire combs were then incubated for 2–3 days in Protein A (P-6031, Sigma) at 250 μg/ml in PBS. The Protein-A-coated nitrocellulose was then washed in 1% PBS/BSA/Tween for 2 h at room temperature.

Serum samples were diluted 1/50 in a buffer consisting of 0·1 M glycine and 0·16 M NaCl adjusted to pH 7·0 with NaOH to reduce aggregation of IgG (Hansson, 1970). Aliquots of 0·25 ml were placed in flat-bottomed microtitre wells in triplicate. The Protein-A-coated combs of nitrocellulose were placed in these wells and incubated at room temperature for 4 h with occasional agitation. Then the combs were removed from the well, washed twice in 1% BSA, 0·05% Tween 20 in PBS (PBS/BSA/Tween), once in PBS, and fixed in 0·5% glutaraldehyde in PBS for 30 min at 0°C. Fixed combs were washed again in PBS at 4°C containing 0·1 M lysine, then boiled for 5 min in PBS in a double waterbath to denature the IgG.

The nitrocellulose was then incubated on a rocking platform at room temperature for 3 h in biotinylated anti-GlcNAc GN7 at a dilution of 1/2000 in PBS/BSA/Tween. After careful washing the nitrocellulose was incubated for 2 h at room temperature in an avidin/peroxidase complex (Amersham) diluted 1/500 in PBS/BSA/Tween.

Subsequently the binding of peroxidase was revealed with a conventional mixture of hydrogen peroxide and precipitating chromogen (4-chloronaphthol, Sigma) in 5 mM Tris/HCl buffer at pH 7·6 for 15 min. After drying the tests were read with a transmitted light photometer adapted from a simple ELISA reader described previously (Rook & Cameron, 1981).

Assay of soluble IL-2 receptors

This was a solid-phase enzyme-linked immunoassay based on two monoclonals recognizing different epitopes (anti-TAC, and 7G7/B6). The antibodies and the assay have been described previously (Rubin *et al.*, 1985; Tung *et al.*, 1987). Results are expressed as arbitrary units/ml based on a standard curve derived from the cell free supernatant of an IL-2-dependent T cell line.

Calculation of results

A curve-fitting program (Dataplot, by S.M. Fraser, Strathclyde University, Glasgow, UK) was used to plot the absorbance values, yielded by the standards in the immunoassay, against the %GO results previously determined biochemically. A log-linear correlation was found ($r=0·94$). The same program was then used to calculate correlations, and to interpolate values for the unknown tuberculosis and leprosy sera.

RESULTS

Correlation between the 'dipstick' immunoassay and the biochemical determination of % agalactosyl IgG (%GO)

The results obtained in the two assays with the set of standards used in this study are shown in Fig 1. The two assays correlate strongly ($r=0·94$) although the biochemical procedure is measuring the number of oligosaccharide chains which terminate exclusively in GlcNAc (i.e. bivalent), while the immunoassay is measuring the presence of an increased percentage of oligosaccharides with one or two terminal GlcNAc's. Such discrepancies as are seen may be due to the presence of some oligosaccharides which lack both Gal and GlcNAc (Parekh *et al.*, 1985) and therefore register as agalactosyl in the biochemical assay, but do not bind the monoclonal, and to a minor but variable contribution from Fab-associated *N*-linked oligosaccharides.

Increased %GO in tuberculosis

Values for the tuberculosis sera were plotted against the age of the donor (data not shown). Values for %GO for 26 of the 30 donors fell more than two standard deviations above the

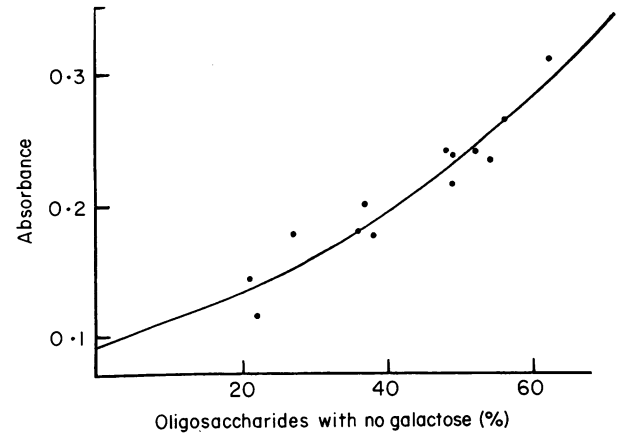

Fig. 1. Correlation ($r=0·94$) between the absorbance obtained using the dipstick assay with whole serum, and the analysis of the oligosaccharides separated from purified IgG. Each point represents a single donor.

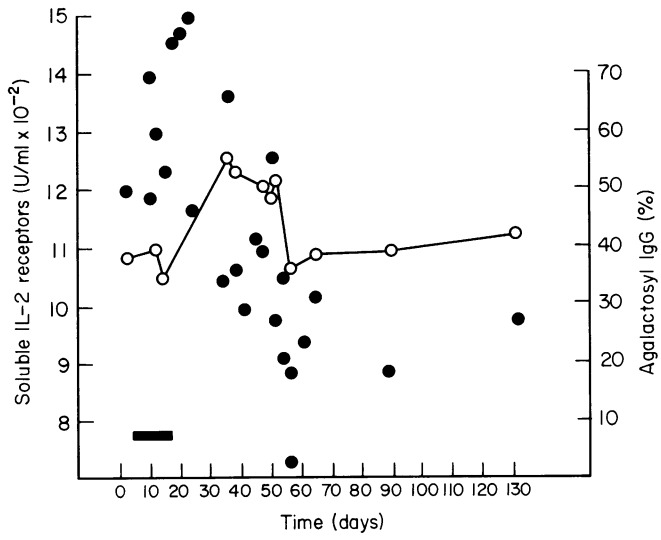

Fig. 2. The rise in soluble IL-2 receptors (●) and agalactosyl IgG (○) in sequential serum samples from a patient before, during and after an episode of ENL (represented by solid bar).

mean value expected for normal donors of the same age (taken from Parekh *et al.*, 1988), confirming the previous finding that %GO is strikingly raised in tuberculosis (Rademacher *et al.*, 1988). Correlations between GO and clinical parameters in tuberculosis are being studied.

Changes in %GO and soluble IL-2 receptors during ENL

Patient 1081/81 was admitted without overt ENL, at which time %GO was between 35–38%. He then developed ENL, and there was a striking rise in soluble IL-2 receptors followed by a rise of GO, reaching a peak of 56% (Fig. 2). Patient 2834/79 was admitted with ENL, at which time his %GO was 63% (Fig. 3). This rose to a peak of 72% but fell during treatment with thalidomide, reaching a trough in the normal range of 33%. It then began to rise again, and ENL recurred and was treated with prednisolone. Several weeks later the patient returned with %GO values varying between 56%–73%, and again suffering from ENL. The data for IL-2 receptors show a very similar pattern (Fig. 3). The other patients showed the same phenomena (data not shown).

A further illustration of the relationship between %GO and free IL-2 receptors is seen in Fig. 4 which shows the correlation between the two assays for a patient (301/80) who suffered repeated ENL episodes and remissions over a period of 40 days.

The peak GO values seen during ENL, and the trough values seen during remission for each of the seven patients are indicated in Table 1.

DISCUSSION

ENL has been considered to be a syndrome mediated by immune complexes (Waters, Turk & Wemambu, 1971). However, changes in T cell function occur during ENL episodes. The helper/suppressor T cell ratio increases, and there is a transient increase in responsiveness of peripheral blood lymphocytes to phytohaemagglutinin and to mycobacterial antigens (Modlin *et al.*, 1986; Rao & Rao, 1987). The present findings confirm a previous observation that there is an increased level of circu-

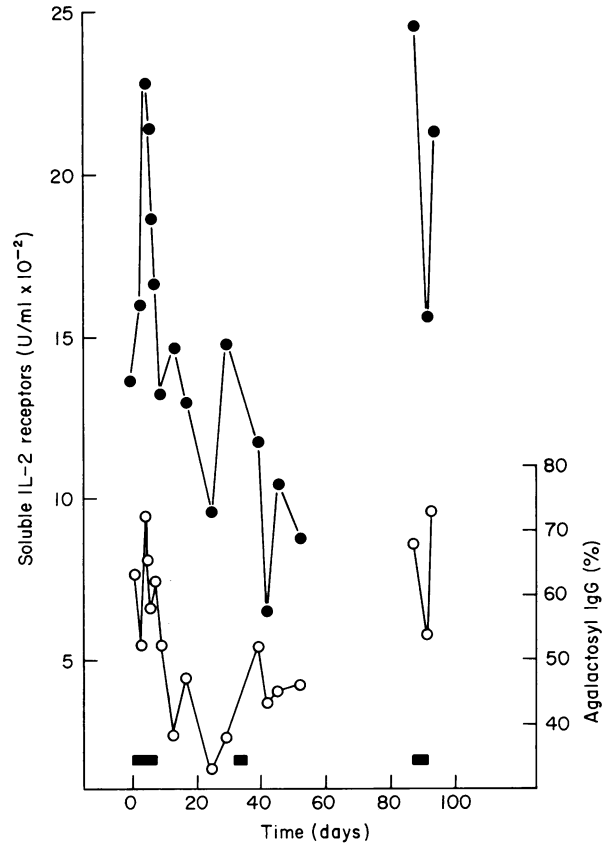

Fig. 3. The rise in soluble Il-2 receptors (●) and agalactosyl IgG (○) in sequential serum samples from a patient before, and during three episodes of ENL (represented by solid bars).

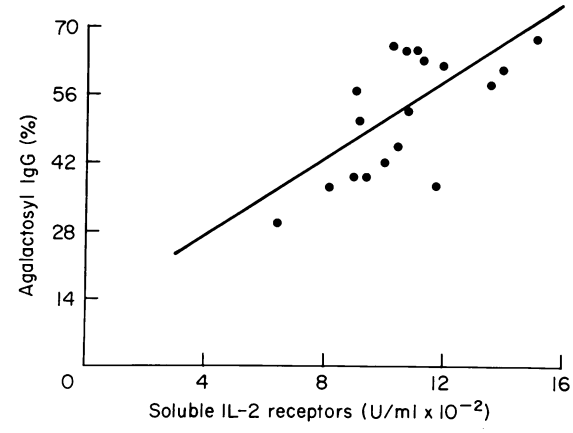

Fig. 4. The correlation between agalactosyl IgG and soluble IL-2 receptors in sequential serum samples from a patient who underwent a series of ENL episodes and remissions over a period of 4 weeks.

Table 1. The lepromatous leprosy studied

Patient	Age (years)	Sex	BI	MI	Type of ENL			Percentage agalactosyl IgG (GO)	
					Fever	Nodules	Joints	Remission	ENL*
301/80	NK	M	3·5	0	+	NK	+	30	72
914/81	NK	M	4·0	0	+	+	−	24	59
3663/80	31	F	1·3	0	+	+	−	13	30
2213/78	40	M	NK	NK	+	+	+	26	45
2834/79	30	M	3·8	0	+	+	±	33	72
1413/81	31	F	4·3	1·2	+	+	+	36	62
1081/81	26	M	NK	NK	−	+	−	35	56

* Maximum value seen during an ENL episode.
NK = not known.

lating IL-2 receptors during ENL, which also suggests increased T cell activity, though it is conceivable that these receptors could be derived from some other cell type (Tung *et al.*, 1987).

The increased level of agalactosyl IgG during ENL episodes indicates that regulatory events are also affecting B cell function since it is likely that agalactosyl IgG increases when the level or activity of β-galactosyl transferase in the B cells is reduced (Axford *et al.*, 1988). It is not clear what regulates this enzyme, though the data presented here provide some clues. Agalactosyl IgG is raised in tuberculosis (Tb), Crohn's disease (CD), and rheumatoid arthritis (RA), all of which are characterized by an acute phase response, but not in leprosy (lepromatous or tuberculoid) without ENL, or in sarcoidosis (Rademacher *et al.*, 1988). This is circumstantial evidence that it only rises during chronic T-cell-mediated inflammation accompanied by a simultaneous acute phase reaction. This concept is strengthened by the observation that in CD, levels of agalactosyl IgG correlate strongly with levels of C-reactive protein (Dube, Rook *et al.*, in preparation). Moreover an acute phase response alone does not evoke agalactosyl IgG since it does not rise in several virus infections (Rademacher *et al.*, 1988) or in rheumatic fever (Bahr, Parekh, Steele *et al.*, in preparation).

It is probable that agalactosyl IgG can contribute to immunopathology since the glycosylation of the Fc of IgG is relevant to function (Nose & Wigzell, 1983; Leatherbarrow *et al.*, 1985; Malaise *et al.*, 1987; discussed in Rademacher *et al.*, 1988). Moreover, agalactosyl IgG has an increased tendency to form aggregates which may themselves be pharmacologically active, and preliminary data from our own laboratories suggest that the glycoforms differ in their tendency to trigger release of cytokines such as tumour necrosis factor from activated macrophages.

The regulatory event which leads to increased agalactosyl IgG, and the pathogenetic significance of its presence are currently of unusual interest in relation to the aetiology of RA. Changes in the level of agalactosyl IgG occur not only in RA and Tb, but also in adjuvant arthritis induced by injecting mycobacteria in oil into rats (Ayala Frenkel, Weizmann Institute, personal communication). This model of arthritis can be transferred to irradiated normal rats with a T cell clone responsive to the 65 kD mycobacterial stress protein (Van Eden *et al.*, 1988) and RA patients have raised levels of antibody to this molecule (Bahr *et al.*, 1988; Tsoulfa *et al.*, 1989). These studies have been discussed recently (Rook, 1988).

The true significance of these relationships is unknown at present. However, arthritic symptoms regularly accompany ENL, and in some circumstances these can progress to a condition closely resembling RA (Ramu & Balakrishnan, 1968; Atkin *et al.*, 1987). Therefore ENL may provide a model of immunoregulatory events with widespread implications.

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