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Thymoma and hypogammaglobulinaemia with and without T suppressor cells

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SUMMARY

Patients with acquired hypogammaglobulinaemia usually have near normal numbers of B cells and normal T cell function. When hypogammaglobulinaemia occurs in association with thymoma, then B cell numbers have been reported as low, and distinctive T cells are present which inhibit immunoglobulin production by normal cells. It has been suggested that these T cells are responsible for the observed hypogammaglobulinaemia. We report a patient with thymoma and hypogammaglobulinaemia who lacks these distinctive suppressor cells and has normal B cell numbers. It is therefore incorrect to propose a single pathogenic mechanism for hypogammaglobulinaemia in association with thymoma.

Keywords thymoma suppressor T cells hypogammaglobulinaemia

INTRODUCTION

Late onset acquired hypogammaglobulinaemia (AHG) is usually associated with normal numbers of B cells in the peripheral blood (Siegal, Siegal & Good, 1978; Platts-Mills *et al.*, 1981). T helper and suppressor functions assayed on control B cells are apparently normal (De la Concha *et al.*, 1977; Platts-Mills *et al.*, 1981; Brenner *et al.*, 1984). A small subgroup exists in which distinctive, radioresistant, T suppressor cells are able to inhibit immunoglobulin production induced by pokeweed mitogen (PWM) (Waldmann *et al.*, 1974; Siegal *et al.*, 1978). However, a survey of the literature suggests that both distinctive suppressor T cells and low numbers of circulating B cells are regularly found, when hypogammaglobulinaemia occurs in association with thymoma (Waldmann *et al.*, 1975; Litwin & Zanjani, 1977; Asherson & Webster, 1980).

This paper describes two patients with thymoma and hypogammaglobulinaemia: one illustrating the classical pattern of low numbers of circulating B cells and suppressor T cells, while the other has normal B cell numbers and no distinctive T suppressor cells. The existence of these patients raises the possibility that hypogammaglobulinaemia in thymoma may have more than one pathogenesis.

MATERIALS AND METHODS

Patients. Patient JOP (born 1914) was found to have a thymic mass in 1973 following a routine chest X-ray taken during the management of diverticular disease of the bowel. Serum globulins were low at this time (17 g/l, normal range 20–37 g/l), but blood grouping and cross-match revealed a normal isohaemagglutinin pattern. After resection of the lymphoepithelial thymoma she remained

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well until March 1983, when she was admitted for treatment of rectal prolapse. At that time isoagglutinins were absent, and she was found to be panhypogammaglobulinaemic (Table 2). She is now treated with i.m. gamma-globulin 25 mg/kg weekly, and remains well.

Patient JC (born 1927) was found to have a thymoma and pan-hypogammaglobulinaemia (Table 2) in 1974, following recurrent chest infections and polyarthritis. After removal of the lymphoepithelial thymoma, he was started on weekly i.m. gamma-globulin (25 mg/kg) with monthly supplements of i.v. fresh frozen plasma (2 units). On this regime his polyarthritis has resolved and he remains well.

Media. Unless otherwise stated, the culture medium used was essentially that devised by Iscove & Melchers (1978), but contained different quantities of transferrin, delipidated albumin and soybean lipid. Its preparation has been described in detail (Brenner *et al.*, 1983).

Cells and cultures. Mononuclear cells were obtained from the peripheral blood of normals or from hypogammaglobulinaemic patients with thymoma. After defibrination, the blood was layered onto Ficoll-paque (Pharmacia) and centrifuged at 650**g** for 35 min. The cells harvested from the interface were washed twice and adjusted to 5×10^6/ml in RPMI 1640, to which 10% heat-inactivated FCS had been added. Each 5 ml aliquot was incubated at 37°C for 90 min in a 5 cm diameter Petri dish (Nunc). The non-adherent cells were removed gently, washed twice in L-15 medium and adjusted to 3×10^6/ml in L-15+10% FCS. To every 3 ml of this cell suspension, 1 ml of 3% neuraminidase treated sheep red blood cells (n-SRBC) was added.

The cell suspension with n-SRBC was centrifuged at 160**g** for 5 min and left at room temperature for 1 h. The pellet was gently resuspended and layered onto Ficoll-paque and centrifuged for 35 min at 650**g**. The interface cells were washed three times, and used as the non-T preparation. T cells were freed of SRBC by adding ammonium chloride solution. When appropriate the T cells were irradiated (2,000 rad, unless otherwise stated), from a ^{60}Co source at 8 rad/s.

Mononuclear cells were cultured using the hanging drop technique previously described (Brenner *et al.*, 1983). Aliquots (10 μl) of twice the final required concentration of non-T cells were dispensed into Terasaki plates using a repeating Hamilton syringe. Similar aliquots of T cells were added. Finally, the stimulant was added in a 1 μl aliquot, to give a final well volume of 21 μl. The cells were cultured by inverting the Terasaki plates and placing them in humidified boxes in a 5% CO_2 incubator.

Analysis of T and B cell phenotype. Peripheral blood lymphocytes were stained with Becton Dickinson (B-D) monoclonal reagents, Leu 1 (pan-T), Leu 2a (suppressor/cytotoxic), Leu 3a (helper), using standard procedures. After incubation with fluorescein conjugated rabbit anti-mouse Ig (B-D), the cells were analysed on a FACS-2. B cells were stained with fluorescein labelled isotype specific rabbit anti-human Ig (Dako), and similarly analysed.

Mitogens. PWM (Sigma) was obtained in freeze dried form and after reconstitution, stored at −70°C.

Assays. The procedure and controls for the assay of total immunoglobulin have been described (Brenner & Munro, 1981). Briefly, Linbro flexible plates were coated with affinity purified anti-globulins. Non-specific binding was blocked with 0·2% BSA and 0·2% gelatine in PBS, the plates washed and test supernatants added. After washing, radiolabelled anti-IgG or IgM was added. After further washing, the counts bound were measured and the antibody present determined from comparison with standard curves derived from the binding of radiolabelled anti-globulin to purified IgG or IgM.

Factor preparation. This has been described in detail (North & Brenner, 1982), and is only outlined here. T and non-T cells were prepared from normal donors. Irradiated T cells were cultured with autologous non-T cells at a ratio of 2:1 in Iscove's medium with 4 μg/ml PWM. After 48 h, the cells were washed four times in fresh Iscove's medium and recultured for a further 72 h, before harvesting the supernatant factors. The content of PWM was determined by a solid phase radioimmunoassay. If detectable quantitites of PWM were present the SN was passed over an anti-PWM column. All supernatant factor used experimentally, contained <2 ng/ml of PWM (<100 pg/ml in final culture), and was passed through a 0·22 μm Millipore filter before use.

RESULTS

Phenotype of peripheral blood lymphocytes

Table 1 shows the phenotypes of the lymphocytes of the two patients with thymoma and hypogammaglobulinaemia. JAC has reduced numbers of B cells which are all $sIgM^+$. In contrast, JP has normal B cell numbers, although again these were nearly all $sIgM^+$. Both patients had reversal of Leu 3a (helper) and Leu 2a (suppressor/cytotoxic) cell ratios. Table 2 records the serum Ig levels of both donors at diagnosis.

B cell function

B cell function was tested using separated T and non-T cells cultured either alone or with factors that induce B cell differentiation in a partially T-dependent manner (North & Brenner, 1982; Brenner *et al.*, 1983). The response of a normal subject is shown for comparison (Table 3).

JAC B cells gave virtually no immunoglobulin response after adding factor either with normal (control) or autologous T cell help. In contrast, JP B cells responded with increased immunoglobulin production on adding B cell differentiation factors, although these cells showed only a low background production of IgM in the absence of such factors. Nonetheless the amount of IgM produced in response to factor was lower than normal, and there was no corresponding rise in IgG.

T cell function

T suppressor cell function was tested by adding patients' irradiated T cells to a co-operating system of normal T and B cells which produced Ig in response to B cell differentiation factors (Table 4). JAC T cells strongly suppressed in this system. In contrast JP T cells failed to suppress. T cells from JP were also tested for their ability to provide help to normal B cells. Thus Table 4 also shows that JP T cells, but not JAC T cells, provide help for IgM and IgG production by normal B cells.

DISCUSSION

It is well established that normal individuals have T cells that provide help for PWM induced Ig production when present at low T : B ratios, and suppress when present at high ratios (De la Concha

Table 1. T and B cell phenotype

	JAC	JP	Normal
Lymphocyte count	$1{\cdot}6\times10^9$/l	$3{\cdot}65\times10^9$/l	
% cells $SIgM^+$	3	12	(>3·8)
$SIgA^+$	0	3	(>0·9)
$SIgG^+$	0	0	(>0·8)
% cells Leu $3a^+$	28	27	(48–80)
% cells Leu $2a^+$	34	39	(23–44)

Normal ranges from Mitchell *et al.* (1983).

Table 2. Serum immunoglobulin levels at diagnosis

	JAC (g/l)	JP
IgG	0·4	1·0
IgM	<0·1	<0·1
IgA	<0·1	<0·1

Table 3. B cell function

Source of irradiated T cells*	Factor†	JAC (patient) IgM	JAC (patient) IgG	JP (patient) IgM	JP (patient) IgG	BC (normal) IgM	BC (normal) IgG
		(μg/ml)		(μg/ml)		(μg/ml)	
—	—	<0·03	<0·03	<0·03	<0·03	<0·03	<0·03
—	+	<0·03	<0·03	<0·03	<0·03	0·06	0·07
N	−	<0·03	<0·03	<0·03	<0·03	0·26	0·09
N	+	0·09	<0·03	0·74	<0·03	19·05	9·33
P	−	<0·03	<0·03	0·03	<0·03	—	—
P	+	0·05	<0·03	1·66	<0·03	−	−

* Forty thousand irradiated (2,000 rad) T cells were added to 2×10^4 B cells from two thymoma patients and one normal, in 20 μl hanging drops. This table illustrates the effects of culturing thymoma B cells with autologous T cells and with normal T cells. As a comparison, it also shows the Ig produced when normal B cells are cultured with normal T cells in this system. Results are the means of triplicate cultures.

† Differentiation factor (see Materials and Methods) was added at 5% final volume.

Table 4. T cell function

Source of T cells* Normal	JAC	JP	IgM	IgG (μg/ml)
+	−	−	43·66	6·31
−	+	−	0·01	<0·01
−	−	+	23·52	4·37
+	+	−	0·07	0·06
+	−	+	30·90	6·02

* Twenty thousand irradiated (2,000 rad) T cells from JAC (thymoma and suppressor), JP (thymoma no suppression) or a normal donor were cultured with 2×10^4 normal allogenic B cells in 20 μl hanging drops in the presence of differentiation factors (see Materials and Methods) added as 5% of final volume. Results shown are the means of triplicate cultures, and show highly significant suppression by JAC T cells ($P < 0{\cdot}0001$) and no significant suppression by JP T cells.

et al., 1977). Suppression is much less marked if the T cells are first irradiated. Waldmann showed that some patients with hypogammaglobulinemia had T cells which suppressed even when present in small numbers. These distinctive T suppressor cells, unlike their normal counterparts are radioresistant (Waldmann *et al.*, 1974, 1975; Siegal *et al.*, 1978; Brenner & Munro, 1981; Callard, Smith & Beverley, 1982 and Table 4).

Although these suppressor cells only occur in a minority of patients with late onset hypogammaglobulinaemia (Platts-Mills *et al.*, 1981), they have been found in many of the patients with thymoma and hypogammaglobulinaemia (Waldmann *et al.*, 1975, Litwin & Zanjani, 1977). Initially it had been suggested that patients with thymoma and hypogammaglobulinaemia have both low numbers of circulating B cells and distinctive suppressor cells. However, the finding of low numbers of B cells is not universal (Asherson & Webster, 1980), and the present paper illustrates that the occurrence of T suppressor cells is also variable.

There is no evidence that hypogammaglobulinaemia causes epithelial tumours such as thymoma. This makes it likely that the causal association of thymoma and hypogammaglobulinaemia is a consequence of the thymoma inducing the immune deficiency, rather than vice versa. A number of pathogenic mechanisms may underlie this association. The finding of distinctive suppressor cells and the relatively normal retention of B cell function after stimulation with Epstein–Barr (EB) virus has led to the suggestion that T suppressor cells are responsible for both the low B cell numbers and the hypogammaglobulinaemia (Waldmann *et al.*, 1975; Pereira, Webster & Platts-Mills, 1982). The present finding of a patient with thymoma and hypogammaglobulinaemia cannot be explained in this way as the number of B cells is normal, and there are no distinctive T suppressor cells. Moreover, B cell function in this patient as tested by EB virus transformation showed the characteristic pattern of late onset hypogammaglobulinaemia described by Pereira *et al.* (1982), normal IgM production with little or no production of IgG (data not shown).

It is difficult categorically to exclude the role of suppressor cells in blocking IgG production in the patient JP. For example it is possible that the IgM to IgG switch requires genetically restricted T helper cells (Andersson, Schreir & Melchers, 1978), and can be inhibited in turn by genetically restricted T suppressor cells. Such restricted help and suppression could only be detected by mixture experiments using genetically matched siblings. Despite this caveat, it seems likely that there are two separate pathogenic mechanisms in thymoma with hypogammaglobulinaemia: one based on the existence of distinctive radioresistant T suppressor cells with secondary loss of B cells, the other based on alteration of B cell maturation. Although this might be due to a direct effect of a thymus factor, consideration of the diseases associated with thymoma (e.g. myasthenia gravis, pemphigus and pure red cell aplasia) indicates that thymoma can give rise to autoimmune disease (Souadjian *et al.*, 1974). This raises the question of whether the hypogammaglobulinaemia may be caused by an autoimmune process, not involving suppressor T cells. While this might be directed against the B cell itself, an alternative target could be the follicular dendritic cell or other specialized cells which present antigen to B lymphocytes.

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