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Expression and function of membrane attack complex inhibitory proteins on thyroid follicular cells

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SUMMARY

Human thyroid cells are resistant to lysis by the homologous membrane attack complex. By immunohistochemical staining we here show that normal thyroid cells and those in Graves' disease and Hashimoto's thyroiditis express two membrane attack complex-inhibiting proteins, CD59 antigen and membrane attack complex-inhibiting protein/homologous restriction factor (MIP/HRF). *In vitro*, the expression of both molecules was enhanced by interleukin-1 (IL-1), tumour necrosis factor (TNF) and interferon-gamma (IFN-γ) and cytokine-treated thyroid cells were more resistant to lysis by homologous complement. Blocking experiments with monoclonal antibodies against CD59 antigen and MIP/HRF showed that both molecules contributed but CD59 antigen was the more important in mediating resistance to complement attack. Expression of these proteins may be an important determinant of the severity of tissue injury produced by complement-fixing thyroid peroxidase antibodies in autoimmune thyroid disease.

INTRODUCTION

Antibodies against thyroid peroxidase (TPO; previously known as the microsomal antigen) are found in most patients with Graves' disease and Hashimoto's thyroiditis.[1] They have been shown to bind to thyroid follicular cells *in vitro* and to fix complement, properties not shared by antibodies against thyroglobulin.[2–4] In Graves' disease, the damage produced by TPO antibodies (and possibly other intrathyroidal destructive processes) is masked initially by the stimulatory activity of antibodies against the thyroid-stimulating hormone (TSH) receptor.[1] However, hypothyroidism is a frequent late outcome in Graves' disease.[5,6]

Several lines of evidence support a major role for complement-mediated cell damage in thyroid autoimmunity. Immune complexes associated with complement deposition are present on the thyroid follicular basement membrane[7] and terminal complement complexes (TCC) have been demonstrated by immunohistochemistry around the thyroid follicles in Hashimoto's thyroiditis and Graves' disease, but not in normal glands.[8] Serum TCC concentrations are raised in these disorders and return to normal after anti-thyroid drug treatment in Graves' disease.[8] We have further shown that cultured human thyroid cells are relatively resistant to lysis by homologous complement, although the formation of sublethal amounts of membrane attack complexes (MAC) impairs the response of these cells to TSH.[9]

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Such homologous restriction of the cytolytic activity of the complement system is due, at least in part, to at least two separate proteins which act at the final stages of MAC assembly. The first to be delineated, MAC-inhibiting protein/homologous restriction factor (MIP/HRF), has a molecular weight of 65,000 and appears to restrict incorporation of C9 into the MAC.[10–12] The tissue distribution of this molecule is currently unclear. The second restriction factor, CD59 antigen, also known as P18, HRF20 or MIRL,[13–16] is an 18,000–20,000 MW protein which acts by preventing C5b-8-mediated insertion and polymerization of C9 in cell membranes.[17,18] In light of our findings with complement-attacked thyroid cells *in vitro*,[9] the aim of this study was to assess the presence and function of MIP/HRF and CD59 antigen on human thyroid follicular cells.

MATERIALS AND METHODS

Purified reagents and antisera

Normal human serum (NHS) was obtained from volunteers and stored in aliquots at -70° until use. Human C9 and C9-depleted NHS were prepared as described previously.[19] Antiserum against human 5′-nucleotidase was provided by Dr J. P. Luzio (University of Cambridge, U.K.). Monoclonal anti-CD59 A35 was the gift of Dr Alex Davies, Dept. of Medical Biochemistry, University of Wales, U.K. and YTH 53.1 was the gift of Professor H. Waldmann, Dept. of Pathology, University of Cambridge, U.K. Anti-MIP/HRF (G3) was prepared as des-

cribed previously.[12] Recombinant interleukin-1 (rIL-1), recombinant tumour necrosis factor (rTNF) and recombinant interferon-gamma (rIFN-γ) were from Boehringer Mannheim (Lewes, U.K.), Biogen (Geneva, Switzerland) and Genentech (San Francisco, CA) respectively. T-cell-conditioned medium (CM) was made by stimulating peripheral blood mononuclear cells (10^7/ml) with 20 μg/ml Concanavalin A (Sigma, St Louis, MO) for 2 days; the supernatant was harvested and free lectin bound with 10 mg/ml α-methyl mannoside (Sigma). Bovine TSH was obtained from Armor (Kankakee, WI).

Cell culture

Human thyroid follicular cells were prepared as detailed elsewhere.[20] Briefly, thyroidectomy specimens from patients with Graves' disease or toxic multinodular goitre were minced and digested with collagenase/dispase; semi-intact follicles were cultured in RPMI-1640 (Gibco, Grand Island, NY) with 10% foetal calf serum (FCS) for 72 hr, with extensive washing to remove non-adherent cells. The thyrocytes were removed with trypsin and stored frozen in liquid nitrogen. For use, the cells were rapidly thawed and recultured in 24-well plates, 10^5/well.

Localization of MIP/HRF and CD59 antigen

Thyroidectomy specimens were obtained from five patients with Graves' disease, one with Hashimoto's thyroiditis and three with benign thyroid nodules (none of whom had thyroglobulin or TPO antibodies). In the latter, normal tissue surrounding the nodules was sampled. The specimens were used for immunohistochemical localizations on 5-μm cryostat sections, as described previously.[8] Briefly, monoclonal anti-CD59 or anti-MIP/HRF (20 μg/ml) were added to the sections, with the monoclonal antibody Ox8 (against a rat T-cell antigen) as a control. Staining was completed by three 10-min alternate cycles of anti-mouse/rat IgG and alkaline phosphatase anti-alkaline phosphatase followed by substrate.[8] Sections were lightly counterstained with haematoxylin.

Western blotting of thyroid cells

Thyroid cells (10^6 cells) from four different glands were washed in phosphate-buffered saline (PBS) and resuspended in 0·25 ml PBS containing 1% CHAPS. After mixing for 1 hr at 4°, the insoluble material was pelleted by centrifugation at 5000 ***g*** in a benchtop microfuge and run on 10% or 15% SDS–PAGE gels under non-reducing conditions in a BioRad minigel system. Gels were then blotted onto nitrocellulose membrane using a BioRad electroblotter (BioRad, Richmond, CA), the membrane blocked with 1% bovine serum albumin (BSA) and then probed with the appropriate antibody—anti-MIP/HRF (G3) or anti-CD59 (A35)—at 10 μg/ml in PBS/0·1% Tween. After washing the blots were developed with anti-mouse IgG HRP conjugate followed by HRP substrate (both from BioRad). Molecular weight markers were run on all gels and used to estimate the relative molecular mass of the blotted proteins.

In vitro localization was performed with thyroid cells which were removed from culture wells using PBS containing 10 mM HEPES and 3 mg EDTA/ml, pH 7·4. Cells were dispersed by gentle pipetting and resuspended in PBS with 0·1% (w/v) BSA and 0·01% (w/v) sodium azide. They contained more than 95% cells staining with the monoclonal antibody 4F2, which reacts with thyroid cells but not fibroblasts.[20] After incubation with anti-CD59, anti-MIP/HRF or Ox8 (20 μg/ml), the cells were washed three times and stained with rabbit anti-mouse/rat IgG-fluorescein conjugate (Sigma). After further washing, flow cytometry on 10^4 cells was performed using an Epics Profile (Coulter, Luton, U.K.).

Complement attack on thyroid cells

Cells in suspension were attacked utilizing classical pathway activation. Thyroid cells (2×10^6/ml) were incubated with anti-5′-nucleotidase antiserum (1:50 dilution) for 5 min at 37°, washed and divided into 100 μl aliquots containing 2×10^5 cells. These were incubated with 500 μl C9-depleted NHS for 15 min at 37°, washed once and then incubated for 15 min at 37° with monoclonal antibodies against MAC-inhibiting proteins (20 μg/ml) as indicated. After further washing, C9 (10 μg/ml) was added and the cells incubated for 15 min at 37°. Cell lysis was estimated by incubation with 0·2% (w/v) trypan blue and the percentage of at least 500 cells staining blue was determined with the observer unaware of the sample's prior treatment.

RESULTS

Expression of CD59 antigen and MIP/HRF

All thyroid glands were stained with monoclonal anti-CD59. This was localized particularly to the apical border of the thyroid follicles, although staining was also evident at the base of the epithelial cells (Fig. 1a). Reactivity appeared stronger on the sections from Graves' and Hashimoto's patients. Staining was much weaker with monoclonal anti-MIP/HRF. Readily identifiable reactivity was only found in thyroids from three of the Graves' patients and one each with Hashimoto's thyroiditis or a benign nodule: in the remaining sections it was difficult to be certain whether binding was greater than background. Again, staining was present on the apical and basal surfaces (Fig. 1b).

Cultured thyroid cells expressed both CD59 antigen and MIP/HRF and this was enhanced by treatment with rIL-1, rIFN-γ and rTNF, as well as T-cell-CM. Representative results and flow cytometer profiles are shown in Table 1 and Fig. 2 using cells derived from a patient with Graves' disease. Similar results were obtained using thyroid cells from two other Graves' patients and one with a toxic multinodular goitre. Expression of CD59 antigen, in the absence of cytokines, increased with the duration of culture. TSH increased expression after 1 day but decreased it after 3 days of culture (Table 2). This was also found in three further experiments with different thyroid cell preparations. In two of three thyroid cell cultures, TSH also enhanced MIP/HRF expression after 1 day but not 3 days of culture (Table 2); in the third experiment, also using Graves' thyroid cells, MIP/HRF expression (both percentage of cells positive and mean fluorescence intensity) was enhanced by TSH after 1, 2 and 3 days culture.

Western blotting of thyroid cell membranes

CD59 antigen and MIP/HRF were readily detected on thyroid cell membranes following SDS–PAGE and western blotting. The estimated molecular weight of CD59 antigen was between 20,000 and 25,000, a similar molecular weight range to that

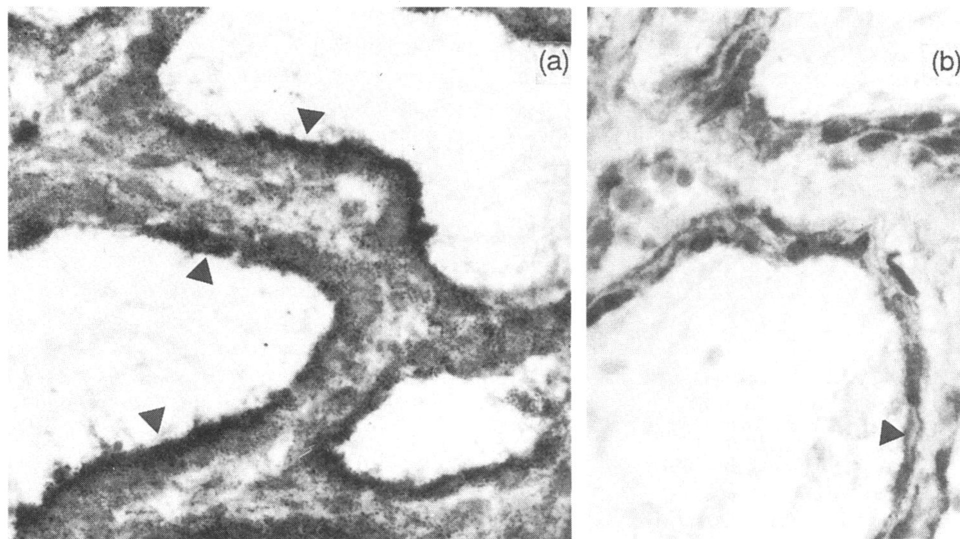

Figure 1. Immunohistochemical staining for (a) CD59 antigen and (b) MIP/HRF on thyroid sections from a patient with Graves' disease. Photomicrographs were taken using a blue-green filter so that positive (red) staining appears dark (arrowed). Original magnification ×400.

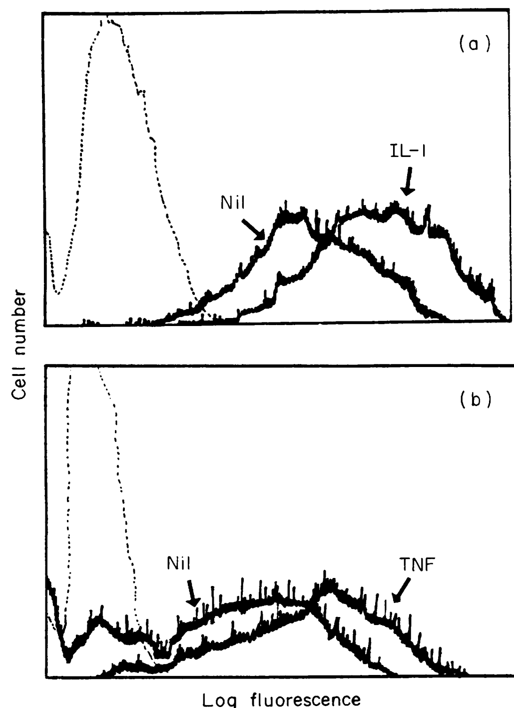

Figure 2. Flow cytometer profiles for thyroid cells stained with (a) anti-CD59 and (b) MIP/HRF. The dotted line represents staining with the control monoclonal antibody, Ox8. Thyroid cells were cultured for 3 days alone (Nil) or with IL-1 (10 U/ml) or TNF (10 ng/ml) as indicated.

found for erythrocyte CD59 antigen (Fig. 3a). Thyroid cell MIP/HRF had a molecular mass of 65,000 under non-reducing conditions, again close to the reported size of erythrocyte MIP/HRF under similar conditions (Fig. 3b). Although the intensity of staining varied between the four glands tested (in Fig. 3a, lane 1 contained a visible band which reproduced poorly) for both MIP/HRF and CD59 antigen, each gland gave bands of the same size for each of these antigens. Further experiments with three further thyroid specimens gave identical results.

Effect of blocking MAC-inhibiting proteins on thyroid cell lysis

An initial series of experiments was conducted to verify the relative resistance of thyroid cells to lysis by homologous complement (Table 3). Fewer thyroid cells were killed with NHS than with guinea-pig serum as a source of complement. Thyroid cells cultured with rIFN-γ plus rTNF showed less lysis with homologous complement than cells cultured for 3 days in the absence of these cytokines. In a total of eight further experiments, using T-cell-CM as a source of cytokines, there was a mean percentage reduction of 10·5% in thyroid cell lysis after treatment with 10% T-cell-CM ($P < 0{\cdot}02$ compared with lysis of untreated cells; paired *t*-test).

The effect of blocking CD59 antigen and MIP/HRF was tested by incubating the cells with the appropriate monoclonal antibodies after the formation of C5b-8 sites on the cells and prior to adding C9. The results of five separate experiments are shown in Table 4. Addition of anti-MIP/HRF led to increased killing of unstimulated thyroid cells from patients 1, 4 and 5, and of thyroid cells from patients 2 and 3 when they were cultured for 3 days with 10% T-cell-CM. The effects of anti-CD59 in increasing lysis were generally more impressive than anti-MIP/HRF, particularly with thyroid cells from patients 2 and 3. The addition of both monoclonal antibodies further enhanced complement-mediated killing with cells from patients 4 and 5.

DISCUSSION

These results demonstrate the presence of MAC-inhibitory proteins, CD59 antigen and MIP/HRF, on thyroid cells *in vivo* and *in vitro*. Although by immunohistochemistry these proteins were present on the apical and basal borders of the thyroid cell, the abundance of CD59 antigen and MIP/HRF was much greater at the apical border. This is where TPO is located and

Table 1. Expression of CD59 antigen and MIP/HRF by Graves' thyroid follicular cells. Cells were cultured for 3 days before assay

Addition	CD59 antigen		MIP/HRF	
	% cells positive*	Mean fluorescence intensity†	% cells positive*	Mean fluorescence intensity†
Nil	58	36·8	45·5	15·7
T-cell-CM (10% v/v)	68·5	49·1	65·6	17·9
rIFN-γ (10 U/ml)	85·0	51·7	75·6	26·2
rIL-1 (10 U/ml)	98·4	88·8	63·0	18·4
rTNF (10 ng/ml)	90·7	45·6	65·6	20·4

* After subtraction of staining with a control monoclonal antibody (Ox8).

† Staining intensity on a logarithmic scale (arbitrary units) after subtraction of staining with a control monoclonal antibody (Ox8).

Table 2. Effect of culture time and TSH on CD59 antigen and MIP/HRF expression by Graves' thyroid follicular cells

Addition	Time (days)	CD59 antigen		MIP/HRF	
		% cells positive*	Mean fluorescence intensity†	% cells positive*	Mean fluorescence†
Nil	1	32·7	24·0	19·8	13·4
TSH‡	1	78·0	61·6	59·4	40·0
Nil	2	52·6	36·6	ND§	ND
TSH	2	31·3	24·6	ND	ND
Nil	3	72·4	55·1	21·2	13·4
TSH	3	18·8	19·9	23·8	13·2

* After subtraction of staining with a control monoclonal antibody (Ox8).

† Staining intensity on a logarithmic scale (arbitrary units) after subtraction of staining with a control monoclonal antibody (Ox8).

‡ 10 mU/ml.

§ ND, not done.

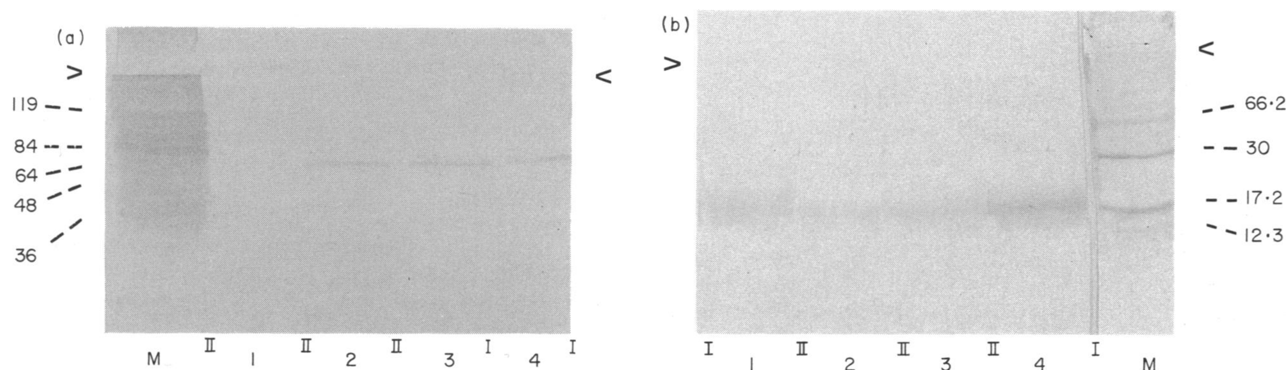

Figure 3. Western blotting of (a) CD59 antigen and (b) MIP/HRF on thyroid cell membranes from one patient with toxic multinodular goitre (lane 1) and three patients with Graves' disease (lanes 2, 3 and 4). The percentages of acrylamide in the gels were 15% for CD59 antigen, and 10% for MIP/HRF. Molecular weight markers (M) were run on each gel. The lane containing the markers was cut off and stained separately in (a) whereas prestained markers were used in (b). The arrowheads in each case represent the top of the running gel.

Table 3. Homologous restriction of complement-induced lysis of human thyroid cells is enhanced by treatment with rIFN-γ plus rTNF

Source of serum	Culture conditions*	% cells killed at various dilutions of serum			
		1:5	1:10	1:20	1:40
NHS	Nil	40	46	25	0
Guinea pig	Nil	>95	86	62	12
NHS	rIFN-γ+rTNF	10	0	0	3

* Cells cultured for 3 days in culture medium with or without rIFN-γ (10 U/ml) plus TNF (10 ng/ml).

where complement-fixing TPO autoantibodies can be detected bound *in vivo* in patients with thyroid autoimmunity,[3] suggesting that the MAC-inhibitory proteins are expressed where they are most likely to function and possibly explaining why previously we have only identified terminal complement complexes at the basal rather than apical portion of thyroid cells in disease.[8] CD59 antigen has been identified on erythrocytes,[14] mononuclear cells,[21] platelets[22] and a number of epithelial as well as endothelial cells,[13,23,24] while MIP/HRF has been demonstrated on erythrocytes,[10,11] platelets, peripheral blood mononuclear cells[24] and amniotic epithelial cells.[25] The molecular weights of the two inhibitors on thyroid cells were similar to those reported for these two proteins on erythrocytes and other cell types.

As complement-mediated injury is important in the pathogenesis of thyroid autoimmunity, protection against complement attack is likely to play a key role in determining the outcome of disease. Of particular interest was the increase in expression of CD59 antigen and MIP/HRF after cytokine treatment. The thyroid mononuclear infiltrate in thyroid autoimmunity is known to produce IFN-γ, IL-1 and TNF[26-28] and these cytokines have been shown to induce the production of other immunologically important molecules by thyroid follicular cells such as major histocompatibility complex (MHC) class II antigens, ICAM-1 and IL-6.[29-31] Cytokine-enhanced expression of MAC-inhibitory proteins may play a role in promoting the resistance of the thyroid cells to antibody-mediated autoimmune damage. This protective role does not necessarily conflict with the effects of these cytokines on MHC class II antigen expression; this too possibly diminishes the autoimmune response through induction of peripheral T-cell tolerance.[32] We also observed that TSH increased CD59 antigen and MIP/HRF expression. This may have pathological relevance as TSH levels are elevated in Hashimoto's thyroiditis and the TSH receptor is excessively stimulated by receptor antibodies in Graves' disease.

The functional effects of CD59 antigen and MIP/HRF were assessed by blocking studies with monoclonal antibodies. Both proteins contributed to resistance to complement attack, but the most impressive effects were seen with anti-CD59 which more than doubled complement-mediated lysis in two experiments. Differences between thyroid cells due to variation in treatment of the patients prior to thyroidectomy, in particular regarding the duration of anti-thyroid drug therapy, may account for some of the inconsistency in the responses seen with blocking antibodies. In four of five experiments, thyroid cells treated with 10% T-cell-CM were more resistant to killing than non-treated cells in the presence of anti-CD59, suggesting that enhanced expression of this molecule prevented complete blocking by anti-CD59: additionally, increased MIP/HRF expression may have contributed.

In summary, these results show that human thyroid cells express both CD59 antigen and MIP/HRF and this expression

Table 4. Effect of monoclonal anti-C59 and anti-MIP/HRF on thyroid cell killing by complement

Exp.	% cells killed after treatment with monoclonal antibodies			
	Nil	Anti-MIP/HRF	Anti-CD59	Anti-MIP/HRF+ anti-CD59
1. NS*	29	39	ND†	38
S*	ND	7	35	30
2. NS	33	29	82	82
S	21	33	44	45
3. NS	29	28	88	89
S	23	29	80	77
4. NS	24	40	35	47
S	20	21	22	25
5. NS	22	31	24	45
S	23	33	32	36
Mean NS(±SD)	27·4±4·4	33·4±5·7	57·3±32·4	60·2±23·4
Mean S(±SD)	21·8±1·5	24·6±11·0	42·6±22·3	42·6±20·6

* NS, non-stimulated—thyroid cells cultured alone for 3 days prior to use; S, stimulated—thyroid cells cultured with 10% T-cell-CM for 3 days prior to use.
† ND, not done.

is enhanced by cytokines which are produced by the lymphocytic infiltrate in thyroiditis. These proteins are important in preventing complement-mediated lysis *in vitro* and are likely to constitute a significant determinant of injury in autoimmune thyroid diseases.

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REFERENCES

1. WEETMAN A.P. & MCGREGOR A.M. (1984) Autoimmune thyroid disease—developments in our understanding. *Endocrine Rev.* **5,** 309.
2. KHOURY E.L., HAMMOND L., BOTTAZZO G.F. & DONIACH D. (1981) Presence of organ specific 'microsomal' autoantigens on the surface of human thyroid cells in culture: its involvement in complement-mediated cytotoxicity. *Clin. exp. Immunol.* **45,** 316.
3. KHOURY E.L., BOTTAZZO B.F. & ROITT I.M. (1984) The thyroid 'microsomal' antibody revisited. Its paradoxical binding *in vivo* to the apical surface of the follicular epithelium. *J. exp. Med.* **159,** 577.
4. ROITT I.M., PUJOL-BORRELL R., HANAFUSA T., DELVES P.J., BOTTAZZO G.F. & KOHN L.D. (1984) Asialoagalactothyroglobulin binds to the surface of human thyroid cells at a site distinct from the 'microsomal' antigen. *Clin. exp. Immunol.* **56,** 129.
5. WOOD L.C. & INGBAR S.H. (1979) Hypothyroidism as a late sequela in patients with Graves' disease treated with antithyroid drugs. *J. clin. Invest.* **64,** 1429.
6. TAMAI H., KASAGI K., TAKAICHI Y., TAKAMATSU J., KOMAKI G., MATSUBAYASHI S., KONISHI J., KUMA K., KUMAGAI L.F. & NAGATAKI S. (1989) Development of spontaneous hypothyroidism in patients with Graves' disease treated with antithyroid drugs: clinical, immunological and histological findings in 26 patients. *J. clin. Endocrinol. Metab.* **69,** 49.
7. KALDERON A.E. & BOGAARS H.A. (1977) Immune complex deposits in Graves' disease and Hashimoto's thyroiditis. *Am. J. Med.* **63,** 729.
8. WEETMAN A.P., COHEN S.B., OLEESKY D.A. & MORGAN B.P. (1989) Terminal complement complexes and C1/C1 inhibitor complexes in autoimmune thyroid disease. *Clin. exp. Immunol.* **77,** 25.
9. WEETMAN A.P., FREEMAN M. & MORGAN B.P. (1990) Thyroid follicular cell function after non-lethal complement membrane attack. *Clin. exp. Immunol.* **82,** 69.
10. SCHONERMARK S., RAUTERBERG E.W., SHIN M.L., LOKE S., ROELCKE D. & HANSCH G.M. (1986) Homologous species restriction in lysis of human erythrocytes: a membrane derived protein with C8 binding capacity functions as an inhibitor. *J. Immunol.* **138,** 1772.
11. ZALMAN L.S., WOOD L.M. & MULLER-EBERHARD H.J. (1986) Isolation of a human erythrocyte membrane protein capable of inhibiting expression of homologous complement transmembrane channels. *Proc. natl. Acad. Sci. U.S.A.* **83,** 6975.
12. WATTS M.J., DANKERT J.R. & MORGAN B.P. (1990) Isolation and characterization of a membrane-attack-complex-inhibiting protein present in human serum and other biological fluids. *Biochem. J.* **265,** 471.
13. DAVIES A., SIMMONS D.L., HALE G., HARRISON R.A., TIGHE H., LACHMANN P.J. & WALDMANN H. (1989) CD59, an Ly-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J. exp. Med.* **170,** 637.
14. SUGITA Y., NAKANO J. & TOMITA M. (1988) Isolation from human erythrocytes of a new membrane protein which inhibits the formation of complement transmembrane channels. *J. Biochem. (Tokyo),* **104,** 663.
15. HOLGUIN M.H., FREDERICK L.R., BERNSHAW N.J., WILCOX L.A. & PARKER C.J. (1989) Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J. clin. Invest.* **84,** 7.
16. OKADA N., HARADA R., FUJITA T. & OKADA H. (1989) A novel membrane glycoprotein capable of inhibiting membrane attack by homologous complement. *Int. Immunol.* **1,** 205.
17. MERI S., MORGAN B.P., DAVIES A., DANIELS R.H., OLAVESEN M.G., WALDMANN H. & LACHMANN P.J. (1990) Human protectin (CD59), an 18,000–20,000 MW complement restricting factor, inhibits C5b-8 catalysed insertion of C9 into lipid bilayers. *Immunology,* **71,** 1.
18. ROLLINS S.A. & SIMS P.J. (1990) The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. *J. Immunol.* **144,** 3478.
19. MORGAN B.P., DAW R.A., SIDDLE K., LUZIO J.P. & CAMPBELL A.K. (1983) Immunoaffinity purification of human complement C9 using monoclonal antibodies. *J. immunol. Meth.* **64,** 269.
20. WEETMAN A.P., VOLKMAN D.J., BURMAN K.D., GERRARD T.L. & FAUCI A.S. (1985) The *in vitro* regulation of human thyrocyte HLA-DR antigen expression. *J. clin. Endocrinol. Metab.* **61,** 817.
21. HIDESHIMA N., OKADA N. & OKADA H. (1990) Expression of HRF20, a regulatory molecule of complement activation, on peripheral blood mononuclear cells. *Immunology,* **69,** 396.
22. NOSE M., KATOH M., OKADA N., KYOGOKU M. & OKADA H. (1990) Tissue distribution of HRF20, a novel factor preventing the membrane attack of homologous complement and its predominant expression on endothelial cells *in vivo*. *Immunology,* **70,** 145.
23. SIMS P.J., ROLLINS S.A. & WIEDMER T. (1989) Regulatory control of complement on blood platelets. Modulation of platelet procoagulant responses by a membrane inhibitor of the C5b-8 complex. *J. biol. Chem.* **264,** 19228.
24. HANSCH G.M. (1988) The homologous species restriction of the complement attack: structure and function of the C8 binding protein. *Curr. Top. Microbiol. Immunol.* **140,** 109.
25. ROONEY I.A. & MORGAN B.P. (1990) Protection of human amniotic epithelial cells (HAEC) from complement-mediated lysis: expression on the cells of three complement inhibitory membrane proteins. *Immunology,* **71,** 308.
26. MARGOLICK J.B., WEETMAN A.P. & BURMAN K.D. (1988) Immunohistochemical analysis of intrathyroidal lymphocytes: evidence of activated T cells and production of interferon-γ. *Clin. Immunol. Immunopathol.* **47,** 208.
27. TURNER M., LONDEI M. & FELDMANN M. (1987) Human T cells from autoimmune and normal individuals can produce tumour necrosis factor. *Eur. J. Immunol.* **17,** 1807.
28. MIYAZAKI A., HANAFUSA T., ITOH N., MIYAGAWA J., KONO N., TARUI S., KIYOTAKI C. & YOSHIZAKI K. (1989) Demonstration of interleukin-1 on perifollicular endothelial cells in the thyroid glands of patients with Graves' disease. *J. clin. Endocrinol. Metab.* **69,** 738.
29. TODD I., PUJOL-BORRELL R., HAMMOND L.J., BOTTAZZO G.F. & FELDMANN M. (1985) Interferon-γ induces HLA-DR expression by thyroid epithelium. *Clin. exp. Immunol.* **61,** 265.
30. WEETMAN A.P., FREEMAN M., BORYSIEWICZ L.K. & MAKGOBA M.W. (1990) Functional analysis of intercellular adhesion molecular-1-expressing human thyroid cells. *Eur. J. Immunol.* **20,** 271.
31. WEETMAN A.P., BRIGHT-THOMAS R. & FREEMAN M. (1990) Regulation of interleukin-6 release by human thyrocytes. *J. Endocrinol.* **127,** 57.
32. LO D.C., BURKLY L.C., FLAVELL R.A., PALMITER R.D. & BRINSTER R.L. (1989) Tolerance in transgenic mice expressing class II major histocompatibility complex on pancreatic acinar cells. *J. exp. Med.* **170,** 7.