THE THYROID AND AUTOIMMUNITY

Proceedings of the International Symposium on Thyroid and Autoimmunity, Amsterdam, 19-21 March 1986

Editors:

H.A. Drexhage
Department of Pathology
University Hospital of the Free University
Amsterdam, The Netherlands

W.M. Wiersinga
Department of Endocrinology
Academic Medical Centre
Amsterdam, The Netherlands

1986

EXCERPTA MEDICA, Amsterdam - New York - Oxford

Contents

AUTOIMMUNE ASPECTS OF HYPOTHYROIDISM

AUTOIMMUNE ASPECTS OF ENDOCRINE OPHTHALMOPATHY

IMMUNE DYSREGULATION IN THYROID DISEASE

© 1986 Elsevier Science Publishers B.V. (Biomedical Division)
The thyroid and autoimmunity.
H.A. Drexhage and W.M. Wiersinga, eds.

THYROTROPIN AND IgG FROM PATIENTS WITH GRAVES DISEASE INDUCE CLASS-II ANTIGEN ON HUMAN THYROID CELLS.

B. E. WENZEL, R. GUTEKUNST, T. MANSKY, T. SCHULTEK, P. C. SCRIBA
Klinik für Innere Medizin, Med. Universität zu Lübeck; F.R. Germany

INTRODUCTION

We were interested in which potential in vivo regulators of thyroid cell functions could modulate HLA-D expression. We investigated the possibility that there might exist a mechanism to induce HLA-D expression different than the well documented pathway via IFN (1,2,3). Namely, the chronic stimulation of the TSH-receptor and/or the administration of iodine in thyroid monolayers (TM) grown in culture medium with or without serum supplementation was studied. This should clarify the role that polarity of three dimensional structures plays in antigen-presentation in vitro as suggested in vivo (4).

MATERIAL AND METHODS

Subjects: Thyroid tissue from patients with Graves´disease (GD) or non toxic goiter (NTG) was obtained at surgery. GD-patients had TSI -and 6 out of 7 microsomal antibodies.

Thyroid cell monolayers (TM): Thyroid tissue was minced, washed intensively with calcium- and magnesium-free phosphate buffer saline (PBS) and digested enzymatically two times for 1 hour at 37° C with 4 mg/ml collagenase. Thyroid cells were washed twice in PBS containing 10 % fetal calf serum (FCS), separated from erythrocytes and auto-rosettes by density centrifugation, washed, and plated on 8 chamber glass slides (20 x 10^4/chamber) in the appropriate medium.

Cell cultures: Two incubation media were used: Iscove medium with 5 % FCS and glutamine or Iscove supplemented with insulin, hydrocortison, human transferrin, somastatin, glycyl-l-hystidyl-l-lysine acetate and 0,5 % FCS (5H). Cells were allowed to adhere over night to the glass slides. After washing with medium, slides were cultured for a further 4 - 5 days with, without - or combinations of the following agents: PHA (0.5 µg/ml), bTSH (1 - 100 mU/ml), NaJ (0.1 mM) and IgG (0.1 mg/ml).

Indirect Immunofluorescence (IF) After 5 days TMs were washed and incubated with monoclonal antibodies: Tü 22- specific for HLA-DQ,

Tü 35- specific for HLA-DP/DR, Tü 39- specific for HLA-DR/DP (BIO-TEST) , DAKO-DRC1 specific for dendritic reticulum cells, DAKO-T1/T2, specific for T-cells (equivalent to OKT1), DAKO-DR reacting with the ß-chain and DAKO-Macrophage. For staining with Ki 67 (DIANOVA), which is specific for a nuclear proliferation antigen (5), TMs were fixed with acetone. The M-antigen of thyroid cells was stained with inactivated, diluted patients sera (α-M $1:320^2$; negative for α-Tg). Rabbit-antigen-mouse IgG $(F(ab)_2)$-FITC and TRITC conjugated rabbit-anti-human IgG were used as second antibodies. IF was assessed with an Olympus photofluorescence-microscope B-H2.

Further procedures IgGs were prepared by ionexchange chromatography. IgGs from GD patients were TSI and α-M positive, while IgGs from NTG and normals had no autoantibodies as measured by specific ELISAs.

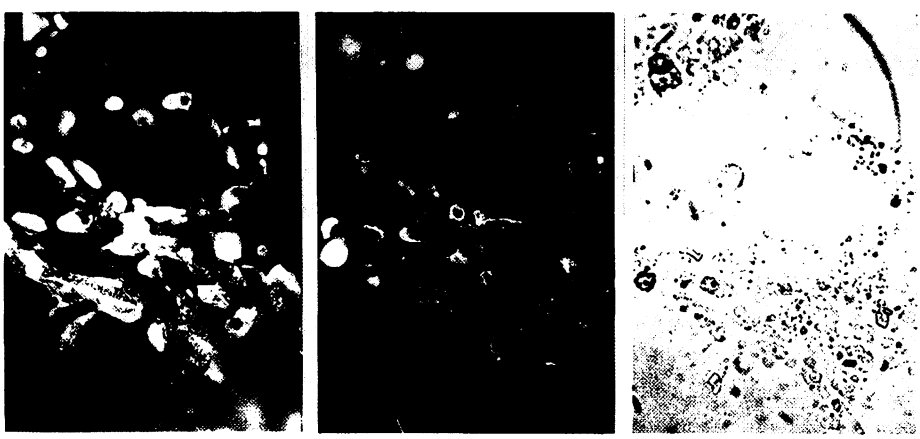

Fig. 1. HLA-DR and M-antigen expressed by thyroid monolayers preincubated with 10 mU/ml bTSH in 5H medium; left: bright light; middle: HLA-DR; right: M-antigen. Original magnification×250

RESULTS

We demonstrated simultanous expression of HLA-DR and microsomal antigen by thyroid cells incubated in serum-free medium with bTSH

TABLE I

Expression of HLA-DR, Microsomal -and Ki 67 antigen in thyroid monolayers derived from GD-patients.

	5H Medium			FCS			
	DR	M	Ki67	DR	M	Ki67	
PHA	+++[a]	0	0	+++	0	0	n=7
TSH	++	+++	+	0	+++	+	n=7
IgG*	++	+++	nd	0	+++	nd	n=3
IgG^{o}	0	+++	nd	0	+++	nd	n=7
IgG^{NTG}	0	0	nd	0	0	nd	n=7
NaJ	0	0	0	0	0	0	n=7
Medium	(+)[b]	(+)	++	0	(+)	+	n=3/7

a - subjectively assessed IF
b - expressed spontanously
* - hyperthyroid GD-patient
o - euthyroid GD-patient
NTG - non toxic nodular goiter
nd - not done

(Fig. 1) or IgG* from hyperthyroid GD-patients (Table I). The HLA-DR expression in TMs was confined to 3 dimensional, follicle-like structures which coincided with the staining of Ki 67, a proliferation antigen (5). This might indicate that chronic stimulation of the TSH-receptor could also induce HLA-DR expression. Moreover, the polarity of the 3 dimensional structures of TMs might account for the absence of HLA-DR in cultures containing FCS (apical up), while DR is present under serum-free conditions (polarity, reversed) (Table I) (6). Since the expression of class II antigen was attributed to contamination of TMs with lymphocytes, we investigated for markers of dendritic, T-cells or macrophages after plating and after 5 days incubation (Table II). Only cultures with FCS showed a weak staining for macrophages or dendritic cells after plating, which disappeared during the incubation period. On the other hand, we could not prove interleukin 1 in TM supernatants. The attempt to block hypothetically produced IFNɣ with cyclosporin A gave inconclusive results. Incubation of TMs with 5 µg/ml cyclosporin A and bTSH detached thyroid cells from slides and neither -DR nor M-antigen was detectable thereafter. Finally, supernatants from TMs displaying -DR spontanously (Tab. I) could not induce -DR on secondary TMs (not shown). In contrast to others,

TABLE II

Expression of HLA-D Polymorphism, Lymphocyte, Macrophage and dendritic cell antigens in thyroid monolayers derived from GD-patients.

		DR	DP/DR	DQ	T1/2	ØM	DRC1
(5H)	PHA	+++	++	0	0	0	0
"	TSH	++	+	0	0	0	0
"	IgG*	++	+	0	0	0	0
"	16h[1]	0	nd	nd	0	0	0
"	5 d[2]	(+)	0	0	0	0	0
(FCS)	16h[1]	0	nd	nd	0	+	+

[1] after plating [2] 5 day incubation

(2,3) we could not find the -DQ loci of HLA-D polymorphism in TMs expressing -DR antigen, althought an induction of class II antigen via IFN would lead preferentially to -DQ expression of TMs(7)

ACKNOWLEDGMENTS

The expert technical assistance of Ms. S. Grammerstorf is greatly appreciated.

This project was supported by "Deutsche Forschungsgemeinschaft" SFB 232/C4.

REFERENCES

1. Todd I, Pujol-Borell R, Hammond LJ, Bottazzo GF (1985) Clin exp Immunol 61: 265
2. Davies TF (1985) J Clin Endocrinol Metab 61: 418
3. Weetmann AP, Volkman DJ, Burman KD, Gerrard TL, Fauci AS (1985) J Clin Endocrinol Metab 61: 817
4. Londei M, Lamb JR, Botazzo GF, Feldman M (1984) Nature, 312: 639
5. Gerdes G, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H (1984) J Immunol, 133: 1711
6. Mauchamp J, Margotat A, Chambard M, Charrier B, Remy L, Michel-Bechet M (1979) Tissue Res, 204: 417
7. Capobianchi MR, Ameglio F, Tosi R, Dolei A (1985) Human Immunology, 13: 1