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Advances in Thyroidology

Cell- and Immunobiological Aspects

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Abstract. The modulation of HLA-D expression of thyroid epithelial cells (TEC) was studied in vitro by means of immunofluorescence. Under serum-free culture conditions, TSH and TSH-receptor antibodies induce HLA-D on TECs derived from GD-patients. Serum-free culture conditions provide a higher availability of TSH-receptors by a 'right side right' polarity of the cellular morphology. There was no evidence for IFN-γ producing cell contaminations on GD-TECs. TSH in contrast to IFN-γ does not induce HLA-DQ on TECs. HLA-DQ is not displayed by spontaneously class-II antigen expressing GD-TECs. Methimazole as well as perchlorate do not suppress HLA-D expression of TECs.

Classically, only immuno-competent cells, namely macrophages or dendritic cells are able to present antigen together with the immuno-regulatory class-II self-antigen (Balfour et al. 1981). These class-II antigens (in man HLA-D locii) can also be found in vivo (Hanafusa et al. 1983) and be induced on thyroid epithelial cells (TEC) by various agents in vitro. In this study 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-γ (Todd et al. 1985; Davies et al. 1985; Weetman et al. 1985). Namely, the chronic stimulation of the TSH-receptor and/or the administration of iodine, methimazole, perchlorate, interferon-γ, TSH-receptor antibodies in TECs grown in culture medium with or without serum supplementation was studied. This aimed at the role that polarity of three dimensional structures play in antigen-presentation in vitro as suggested in vivo (Londei et al. 1984; Wenzel et al. 1986).

Materials and Methods

Patients

Thyroid tissue was obtained from patients with Graves' disease (GD) or non-toxic goitre (NTG). GD-patients were iodine loaded for 10 days before surgery. They also had thyroid stimulating antibodies (TSAb), and 6/7 had microsomal (M-antibodies). Patients with NTG were all void of thyroid antibodies.

Thyroid epithelial cells (TECs)

Thyroid tissue was minced, washed intensively with calcium- and magnesium-free phosphate buffer saline (PBS) and digested enzymatically two times for 1 h at 37°C with 4 mg/ml collagenase. Cells were then washed twice in PBS containing 10% foetal calf serum (FCS), separated from erythrocytes and auto-rosettes by density centrifugation, washed again and plated on 8 chamber glass slides (20×10^4 cells/chamber) in Iscove medium. The medium was either supplemented with insulin, hydrocortisone, somastatin, human transferrin and gly-his-lys three peptide in 0.5% FCS (5H-medium) or with 10% FCS.

Cell cultures

TECs were allowed to adhere overnight to the glass slides. After washing with medium, slides were cultured for a further 4 to 5 days with, without, or combinations

of the following agents: interferon-γ (IFN-γ) 10 U/ml, bovine thyrotropin (bTSH) 1–100 mU/ml methimazole (MMI) 1–100 μM; perchlorate (PC) 1–100 μM; sodium iodide (NaI) 0.1 mM; IgG 0.1 mg/ml. In some experiments supernatants from TECs were collected after 2 days and tested on secondary TEC cultures.

Indirect immunofluorescence (IF)

After pre-incubation TECs 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; DAKO-DRC1 – specific for T-cells (equivalent to OKT1); DAKO-DR reacting with the β-chain and DAKO-macrophage (ØM). The M-angigen of thyroid cells was stained with inactivated, diluted patient's sera (α-M1: 320^2; negative for α-Tg). Rabbit-anti-mouse-IgG ($F(ab)_2$)-FITC and TRITC conjugated rabbit-anti-human IgG were used as second antibodies. IF was assessed with an Olympus photo-fluorescence-microscope B-H2.

Further procedures

IgGs were prepared by ion-exchange chromatography. IgGs from GD-patients were TSab and α-M positive, while IgGs from NTG and normals had no autoantibodies as measured by specific ELISAs.

Materials

Collagenase (Dispase II) was from Boehringer, Mannheim, FRG. Foetal calf serum (FSC), Iscove medium and all cell culture additives were from Biochrom, West-Berlin. Monoclonals, Tü-22/35/39 and interleukin-2 were from Biotest, Dreieich, FRG. FITC and TRITC conjugated second antibodies were from Dakopatt, Hamburg, FRG. Interferon-γ, Methimazole and all hormone additives were from Sigma Chemie, Munich, FRG. Sodium iodine and potassium perchlorate were from E. Merck, Darmstadt, FRG. Thyrotropin (Thyreostimulin) was from Organon, Munich FRG. Micro-Lab slides (Miles) and sheep erythrocytes were from Flow, Meckenheim, FRG. A BH-2 from Olympus Europe, Hamburg, FRG was used.

Results

TECs reassociate in cultures into dome-like structures sometime resembling micro-follicles as shown in Fig. 1. Under low serum conditions (5H-medium) the cellular polarity of these structures appears 'right side right'.

In Fig. 2 this particular morphology is demonstrated by nucleii surrounding a lumen with microvilli and filaments of TECs pointing inside. The ability of various agents to induce class-II depends on the source of the TECs, the agent used, and the polarity of cells in cultures. IFN-γ induced HLA-DR, but not M-antigen regardless

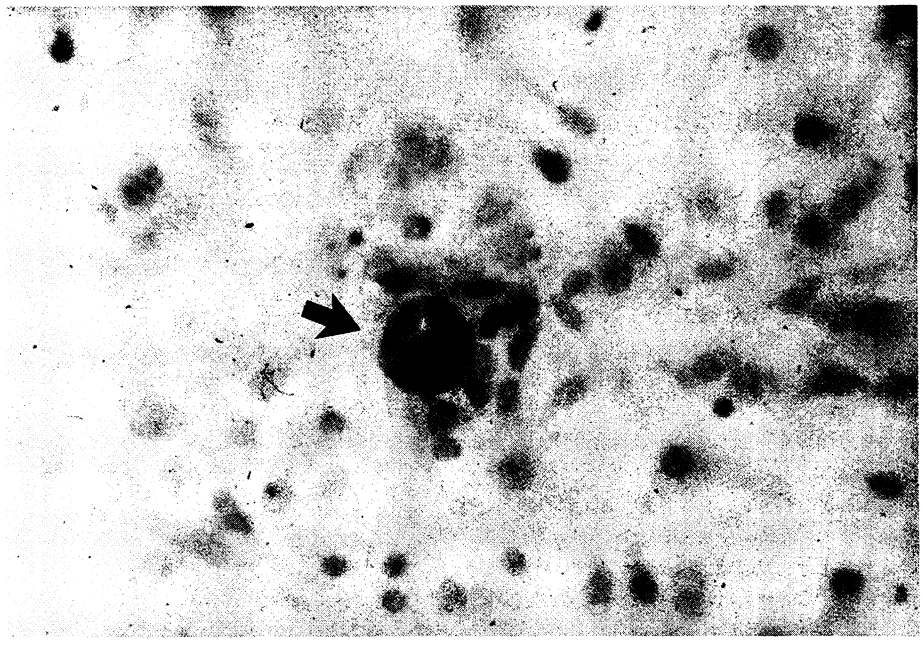

Fig. 1.
Re-association of thyroid epithelial cells under serum-free (5H-medium) culture conditions.

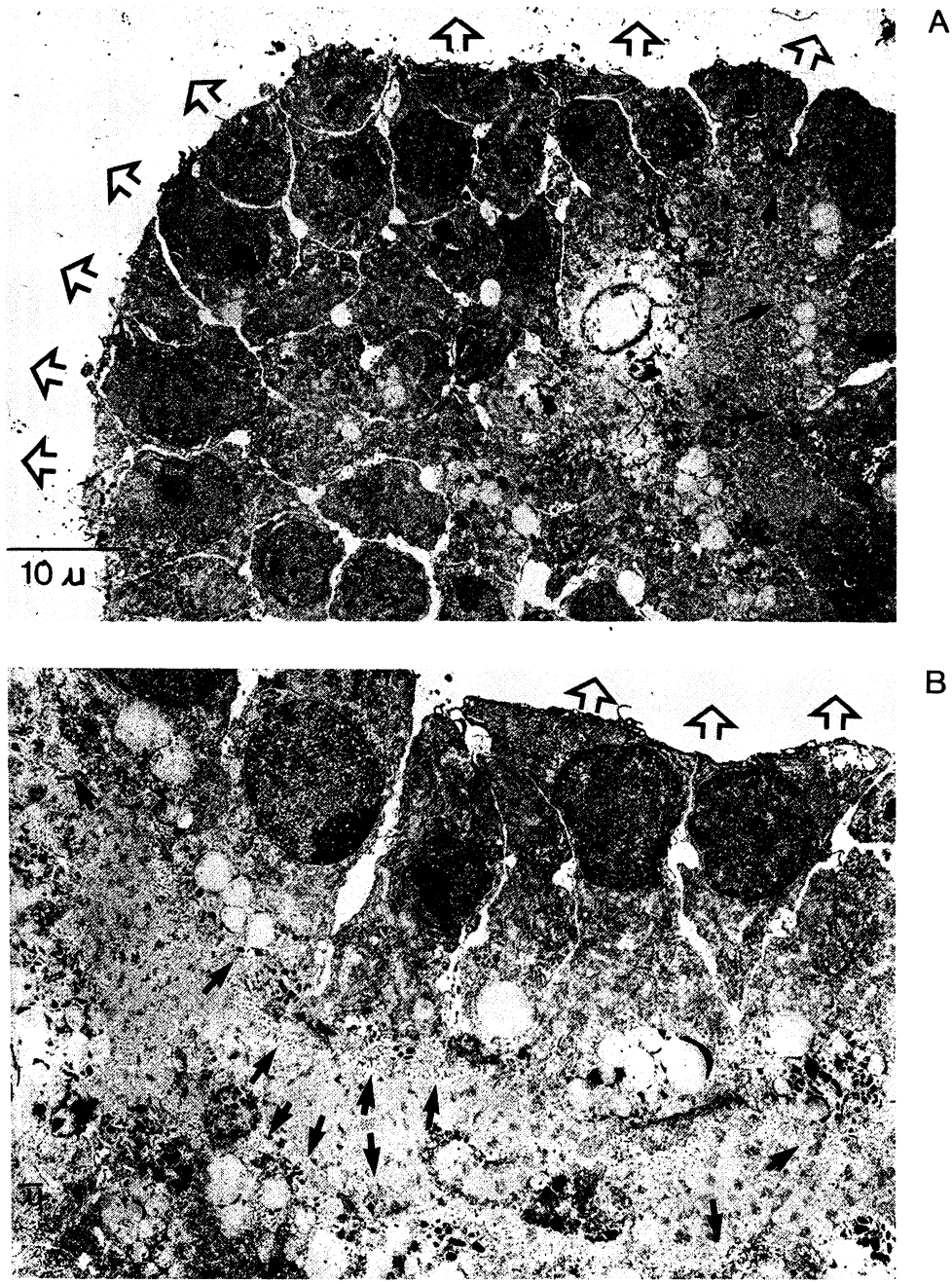

Fig. 2.
Electron-micrograph of follicle-like structures of thyroid epithelial cells grown in 5H-medium. A: Dome-structure; B: Close-up. ⇨ site pointed to culture medium (outside). → microvilli.

of the culture conditions or whether TECs derive from GD-patients or patients with NTG (Table 1).

All agents acting on the TSH-receptor, i.e. TSH and IgG from hyperthyroid GD-patients, induce M-antigen (Table 1). HLA-DR together with M-antigen is only induced in 5H-medium (Fig. 3; Table 1).

A spontaneous expression of HLA-DR was ob-

Table 1.
Expression of HLA-DR and M-antigen by thyroid epithelial cells derived from patients with Graves' disease or with non-toxic goitre.

	Graves' disease				Non-toxic goitre	
	5H		FCS		5H	
	DR	M	DR	M	DR	M
IFN-γ	+++	–	+++	–	+++	–
TSH	++	+++	–	+++	–	+++
IgG*	++	+++	–	++	–	++
IgG^{NTG}	–	–	–	–	–	–
IgG**	–	–	–	–	–	–

* Hyperthyroid GD-patients. ** Euthyroid GD-patients.

Table 2.
Spontaneous expression of HLA-DR by the thyroid epithelial cells derived from hyperthyroid patients with Graves' disease.

	5H (1 day)	5H (5 days)	5H (5 days + TSH)	SN*
HLA-DR	+	–	–	–
M-antigen	++	–	++	–

* Supernatant from spontaneously HLA-DR expressing thyroid epithelial cells applied on secondary NTG-cell cultures for 5 days.

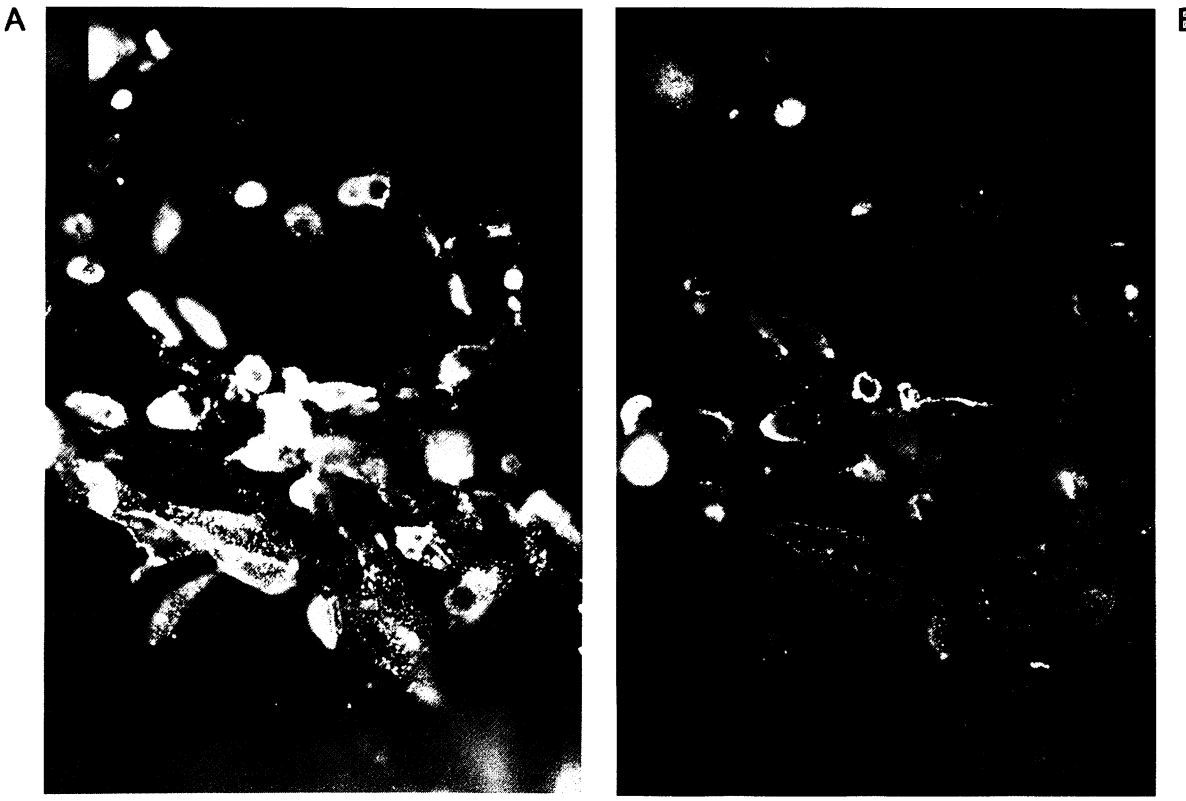

Fig. 3.
Expression of HLA-DR and M-antigen by thyroid epithelial cells from patients with Graves' disease. Cells were incubated with 1 mU/ml bTSH in 5H-medium for 4 days; magnification approx × 450. A: α-M + a-hIgG-TRITC. B: α-HLA-DR + a-mIgG-FITC.

Table 3.
Expression of HLA-D polymorphism, lymphocyte-, macrophage-, and dendritic cell antigens by thyroid epithelial cells from Graves' patients.

	DR	DP/DR	DQ	T1/T2	øM	DRC1
IFN-γ	+++	++	+++	–	–	–
TSH	++	+	–	–	–	–
IgG*	++	+	–	–	–	–
5H (1 day)	+	(+)	–	–	–	–

* Cells grown in 5H-medium.

served in some TEC-cultures derived from GD-patients (Table 2). This disappeared after 5 days in culture. But HLA-DR expression could be re-induced with TSH. Supernatants (SN) of these particular TEC-cultures could not induce class-II expression, when applied on secondary TEC-cultures. When the HLA-D polymorphism of class-II expressing TECs was assessed, different staining patterns were observed with different inducing agents. While TSH, IgG-GD* induced, and spontaneously expressing TECs never displayed HLA-DQ, a bright stain of HLA-DQ was found after IFN-γ incubation (Table 3).

TEC-cultures were investigated by means of IF with specific monoclonal antibodies for contaminations with dendritic cells, macrophages or T-cells. Only in cultures with FCS could a weak staining of scattered macrophages sometimes be observed. When the effect of MMI or PC on IFN-γ or TSH induced HLA-D expression was investigated, only a slight decrease of HLA-D staining could be observed (Table 4).

Discussion

Admittedly, these in vitro studies can only give limited information about the induction of class-II expression by TECs in vivo. Our studies suggest, however, that the chronic stimulation of TECs through the TSH-receptor as well as the cellular polarity of TECs play a role in the modulation of class-II expression. We postulate that the cellular polarity modulates the availability of TSH-receptors in TEC-cultures (Mauchamp et al. 1979). This appears to be the reason, why the induction and reexpression of class-II antigen by TSH could only be observed under serum free culture conditions. The class-II expression of TECs in vivo (Londei et al. 1984) resembles more generally the staining pattern we observed after induction with TSH than that with IFN-γ. In contrast to TEC-cultures expressing spontaneously HLA-DR and M-antigen, IFN-γ strongly induces HLA-DQ, and can induce in vitro TECs derived from autoimmune (GD) as well as non-immune (NTG) thyroid patients. Moreover, we could not detect by means of indirect IF contaminating dendritic cells, T-cells or macrophages which would account for IFN-γ production in our serum free culture system. In addition to that, the supernatants of spontaneously class-II expressing TEC-cultures could not induce class-II in secondary TECs, which one would expect if IFN-γ would be produced by contaminating activated lymphocytes in TECs. On the other hand, we could also

Table 4.
Effect of methimazole (MMI) and perchlorate (PC) on the HLA-DR of thyroid epithelial cells.

	IFN-γ	+MMI	+PC	+TSH	TSH	+MMI	+PC
DR	+++	+++	++	+++	++	++	+
DQ	+++	++	++	+++	–	–	–

not detect interleukin-1 in these supernatants (not shown).

The effect MMI has during suppression therapy of GD in vivo and on antibody synthesis in vitro (McGregor et al. 1980) has been attributed to immuno-suppressive effects. In our hands, MMI and PC have no effect on class-II expression of TECs. This reflects previous findings in recurrent hyperthyroidism of GD-patients (Carel et al. 1986) and findings in GD-therapy where MMI had the same effect as PC, which surely is not considered as an immuno-suppressive drug (Wenzel et al. 1984).

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