THYROID AUTOIMMUNITY

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AN IN VITRO MODEL FOR THYROID AUTOIMMUNITY

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INTRODUCTION

Clinical observations have suggested an association between iodine intake and the occurance of autoimmune thyroiditis in man (1,2), while in areas with endemic goiter prevalence due to iodine deficiency a lower incident of Hashimoto's thyroditis was found (3). Others, however, have contested these claims (4,5). Experimentally, this association has been demonstrated with genetically susceptible chicken (6), rats (7) and dogs (8) on a high iodized diet. In the present study, we were interested in the effect iodine would have on the functional immune response of human T-lymphocytes in co-cultures with autologous thyroid epithilial cells (TECs). Classically, only immunocompetent cells, namely macrophages are able to present antigen together with the immunregulatory class-II surface antigen(9).These class-II antigens (in man HLA-D locii) can also be found in vivo (10) and induced by various agents in vitro(11) on TECs. With this background our in vitro model system was designed to i) modulate HLA-D expression on TECs and ii) to investigate,if a potential iodide induced autoantigen on TECs would be presented through HLA-D to autologous T-lymphocytes and thus initiate a proliferative cellular immunresponse. In order to modulate the hypothetical iodine induced autoantigen the influence of Methimazole (MMI) and perchlorate (PC) on the co-culture system was to be studied. MMI has been suspected to act immune suppressively during suppression therapy of Graves' disease (GD) in vivo and on antibody synthesis in vitro (12). Interestingly, when MMI therapy in GD hyperthyroidism was compared to PC treatment, which clearly is no immuno suppressive agent, both drugs had the same effect on the production of thyroid stimulating antibodies (TSab) (13).

METHODS

Subjects

Thyroid tissue was obtained from patients with GD or non toxic goiter (NTG). GD patients with the exception of two

where in cpm = cpm (TEC + Ly) - cpm (TEC) - cpm (Ly)

Indirect immunoflorescence (IF) (11).

TEC were plated and cultured on 8 chamber glass slides (20×10^3) cells (chamber) in the appropriate medium with or without inducing agents. After 4 days TECs were washed and incubated with specific monoclonal antibodies for HLA-D or M-antigen. Double stained slides were developed with a mouse IgG or a human IgG coupled to FITC or TRITC , respectively.

MATERIALS

Collagenase (Dispase II) was from Boehringer, Mannheim, FRG. Fetal calf serum (FCS), Isco ve (T) 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. Interferonγ, Methimazole and all hormone additives were from Sigma Chemie, Munich, FRG. Sodium iodide and Potassium perchlorate were from E. Merck, Darmstadt, FRG. Thyrotropin (Thyreostimulin) was from Organon, Munich, FRG. MicroLab slides (Miles) Phytohemagglutinin and sheep erythrocytes were from Flow, Meckenheim, FRG. A Cell harvester,Multiwash 2000, Dynatech, Denkendorf, FRG and a Flourescence-Photomicroscope, BH-2 from OLympus Europe, Hamburg, FRG were used.

RESULTS

We demonstrate simultaneous expression of HLA-D and M-antigen, when TECs are preincubated with bTSH in 5H (serum free) medium. In contrast, no M-antigen is re-expressed in TECs after incubation with PHA or IFNγ (Table 1.). The HLA-D

Table 1. In vitro Expression[1] of HLA-D Polymorphism and Microsomal Antigen in Preincubated TEC Cultures.

	DR	DP/DR	DQ	M
PHA	+++	++	0	0
+NaI	++	+	0	0
TSH	++	+	0	+++
+NaI	+	+	0	++
IFNγ	+++	++	+++	0
+NaI	++	+	++	0
NaI	(+)[2]	0	0	0
MMI	(+)	0	0	0
PC	0	0	0	0
Medium	(+)	(+)	0	(+)

1-assessed by IF 2-in some GD-TECs spontaneously

cases were iodine loaded for 10 days before surgery. They also had TSab and 6/7 had microsomal (M) antibodies. Patients with NTG were all void of thyroid antibodies.

Thyroid epithelial cells

Thyroid tissue was minced, washed and digested enzymatically with 4 mg/ml collagenase two times for 90 minutes at 37 C. Cells then were washed, separated from debris and erythrocytes by density centrigfugation, washed again and plated in Iscove medium containing insulin, hydrocortisone, somastatin, human transferrin and gly-his-lis tripeptide in 0,5% fetal calf serum (FCS) (5-hormone,(5H) medium).

Cell Cultures

TECs were allowed to adhere overnight in 250 ml/96 well flat bottom Microtiter plates (20×10^3 cells/well). After washing with medium, TECs were incubated for 4-5 days with Interferon γ (IFN γ) 10 U/ml, Phytohemagglutinin (PHA) 0.5 µg/ml, bovine thyrotropin (bTSH) 1-100 mU/ml together with or without sodium iodide (NaI), 0.1 mM, MMI, 1-100 µM and PC , 1-100 µM.

On day 5 after starting TEC cultures peripheral blood was drawn from the donor of the thyroid tissue. Lymphocytes were prepared by density centrifugation and T-cells were separated by rosetting with sheep red blood cells (SRBC).

When intra-thyroidal lymphocytes were prepared, the initial TEC suspension after collagenase digestion was divided up. One part was used for establishing TEC cultures, while the other part was allowed to adhere overnight, only that thereafter the non-adherend cells were purified by density centrifugation. All cells banding with lymphocytes were washed, frozen and stored in liquid nitrogen until use on the fifth day of autologous TEC cultures. The yield of intra thyroidal T-lymphocytes was very variable. Depending on the source of the thyroid tissue 1-30 million T-lymphocytes could be obtained.

Co-cultures

After the preincubation period for TECs $2\text{-}4 \times 10^5$ T-lymphocytes were added to each Microtiter well ($1\text{-}2 \times 10^5$ cells /ml) using Iscove medium-T supplemented with 15% interleukin 2 (IL2). Co-cultures were incubated for another 6 days, whereafter the 24 hour tritiated thymidin (^{3}HTdR) uptake was measured.

^{3}HTdR uptake

To each Microtiter well 0.5 µCi/50µl ^{3}HTdR (2 Ci/mmol) were added. After 24 hours the non attached cells in co-cultures were resuspended, harvested with a cell-harvester on to cellulose acetate filter, precipitated with 10% tri-chloracetic acid (TCE) and washed with 96% cold ethanol. Filters were dried, punched out and disolved in scintillation cocktail. Samples were set up in quadruplicates and stimulation indices were calculated as:

$$\text{cpm}(+\text{IL2}) / \text{cpm}(-\text{IL2}) = \text{SI} + \text{SD}$$

Table 2. HLA-D Expression and Autologous T-Lymphocyte Response in Co-Cultures with TECs.

	HLA-D	SI ± SD
PHA	& +++	1.5 ±0.4
+NaI	++	5.6 ±1.1 **
+ " +MMI	+	1.1 ±0.6
+ " +PC	+	0.9 ±0.6
IFN γ	+++	1.6 ±0.2
+ NaI	++	5.1 ±0.6 *
+ "+MMI	++	1.8 ±0.5
+ "+PC	+	1.5 ±0.6
TSH	++	1.2 ±0.7
+NaI	+	4.1 ±0.8 *
+ "+MMI	+	0.9 ±0.4
+ "+PC	+	1.0 ±0.3
Medium	-	1.8 ±0.5

& -IF *$p < 0.05$ **$p < 0.01$

inducing agents also differ, when HLA-D polymorphism was assessed. While IFM strongly induces HLA-DQ, PHA and TSH incubated TECs display only DR/DP and no -DQ (Table 1). Although NaI is reducing flourescence stain on induced TECs, there is no suppressing of HLA-D observed. The same applies for MMI and PC at concentration lower than 0.1 mM in cultures. In some TECs from GD-patients a spontaneous HLA-D expression occurs, which disappears after 3 days in culture.

As shown in Table 2.only those co-cultures,where HLA-D was induced together with NaI incubation, autologous T-lymphocytes responded with a significantly increased proliferation. This autologous MLR-likereaction was abolished by simultaneous incubation with MMI or PC. Both agents, however, did not suppress HLA-D expression under the same preincubation conditions.

Table 3. Proliferative Response in Co-Cultures of TECs with Autologous Intra-thyroidal T-lymphocytes from GD-Patients.

	IFNγ	IFNγ+NaI	Con A
Patient 1	§ 1.6 ±0.4	1.3 ±0.5	5.2 ±0.4
Patient 2	1.6 ±0.2	1.6 ±0.5	6.9 ±0.6

§ -SI ± SD

When intra-thyroidal T-lymphocytes were used under identical co-culture conditions, no proliferative response was observed on IFNγ/NaI preincubated TECs (Table 3), although the ConA T-lymphocyte response was still intact and the T-suppressor T-helper ratio were not altered (visually checked by IF).

There appears to be a difference in T-lymphocyte responses according to iodine administration before surgery. Co-cultures of GD-patients which have not been loaded with iodine tend to have lower SI than those of patients "plummered" for 10 days (Table 4.). When co-cultures were set up with TECs from patients with NTG only 4/15 T-cell responses were observed (not shown).

Table 4. The Influence of Iodine Load on the Autologous T-Lymphocyte response in Co-Cultures with TECs from GD-Patients.

	"Plummer"	SI ±SD
Patient 1	no	2.8 ±0.7
Patient 2	no	3.1 ±0.4
Patients, n=7	yes	5.8 ±0.6

" " Iodine loaded for 10 days pre-Op.

DISCUSSION

Our in vitro co-culture system appears to be a model for iodine induced autoimmunity(2).It is comparable to the animal models using obese chicken(6) or BB/W rats(7).Likewise in those models the autologous T-lymphocyte response in our in vitro system is genetically suszeptibility dependent,since 10/10 GD-donors with proliferative responses are contrasted by only 4/15 NTG-patients.Wether this could reflectalternatively the actual iodine deficiency in the goitrous thyroid is not yet understood.One hint in that direction could be the difference in magnitude of the response in iodine loaded and unloaded GD-patients.

In order to obtain a T-lymphocyte response two requirements have to be met: 1.An autoantigen has to be induced.In our model this is apparently iodine induced and dependent,since preventing the iodine uptake with PC abolishes the response. 2. The autoantigen has to be presented to immunocompetent lymphocytes.It appears that the thyrocyte is presenting itŝ own autoantigen via HLA-D,since HLA-D expression of TECs is a condition for the response.This would confirm findings,where class-II antigen expressing epithelial cells were able to present viral proteins(14).MMI does not suppress class-II antigen,which reflects previous findings in recurrent hyperthyroidism od GD-patients(15).
Moreover,the similar behaviour of MMI and PC in our system recalls findings in GD therapy,where both agent had the same

effect on thyroid antibody titers during the course of the disease(13).

Our failure to produce a proliferative response with intrathyroidal T-lymphocytes is puzzling and hard to interpret. Although the Ts/Th ratio and the mitogenic T-lymphocyte response are not altered, there might be a loss of T-helper cells during the preparation procedure.

Ongoing studies in our laboratory are designed to define the iodine dependent autoantigen.

SUMMARY

TSH, in contrast to PHA and IFNγ, induces in serum free medium both, class-II and microsomal antigen. The class-II polymorphism displays DR/DP loci rather than DQ, while IFN always strongly induces DQ. Iodine produces autoantigen in TECs which together with class-II expression gives rise to an autologous mixed lymphocyte reaction -like response. The iodine induced immune response in co-cultures prevails in TECs from GD-patients(10/10), while only 4/15 NTG-TECs gave response. Mehimazole as well as perchlorate abolish the response, although they do not suppress the class-II expression of TECS. Iodine could not induce a proliferative response with intra-thyroidal lymphocytes . The mitogenic T-cell response is not abolished. Responses in iodine loaded patients are higher than in the two GD-patients who were not " plummered" before operation.

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