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Contents

AUTOIMMUNE ASPECTS OF HYPERTHYROIDISM

The role of antibodies and other humoral factors in the pathogenesis of Graves' disease <i>S.H. Ingbar</i>	3
Elisa for autoantibodies reacting with thyroid plasma membrane antigens in serum of patients with autoimmune thyroid diseases in comparison with anti TSH receptor antibodies assay <i>A. Gardas and H. Domek</i>	15
Can porcine thyroid tissue replace human thyroid tissue in the assay of thyroid stimulating antibodies? <i>S. de Rave, H.M.J. Goldschmidt, B. Bravenboer, Y.T.J. Somers-Pijnenburg and J.H.M. Lockefeer</i>	17
Identification of different immunoglobulin G epitopes in Graves' disease <i>A. Garcia-Ameijeiras, A. Duque, E. Rosell and R. Corcoy</i>	19
Isolation of both agonist and nonagonist Graves' immunoglobulins from the sera of individual patients <i>J. Worthington, R.L. Himsworth and P.G.H. Byfield</i>	21
Characterization of TSH receptor antibodies in Hashimoto's thyroiditis and Graves' disease <i>T.W.A. de Bruin, L.E. Braverman and R.S. Brown</i>	23
Thyroid stimulating antibody: An index of thyroid stimulation in Graves' disease? <i>L. Baldet, A.M. Madec, C. Papachristou, A. Stefanutti, J. Orgiazzi and C. Jaffiol</i>	26
Detection of thyroid-stimulating antibodies in Graves' disease and in other thyroid diseases, employing rat thyroid fragment perfusion <i>J.R. Attali, P. Valensi, D. Darnis, C. Weisselberg and J. Sebaoun</i>	29
Thyroid-stimulating immunoglobulins (TSI) and thyroglobulin autoantibodies (h-TgAb) in Graves' disease <i>P.H. de Nayer, P.H. Sauvage, C. Cornette, M.-P. Lambot and C. Beckers</i>	33
Graves' disease; Factors associated with its development and subsequent course <i>D.C.S. Chang, P.S. Barnett, S. Ratanachaiyavong, C.A. Gunn, C. Darke and A.M. McGregor</i>	37
The predictive value of pretreatment TSI measurements in Graves' disease <i>B. Bravenboer, S. de Rave, H.M.J. Goldschmidt, Y.T.J. Somers-Pijnenburg and J.H.M. Lockefeer</i>	50

The ability of the serum thyrotrophin receptor antibody (TRAb) index and HLA status to predict long term remission of thyrotoxicosis following medical therapy for Graves' disease <i>R. Wilson, J.H. McKillop, N. Henderson, D.W. Pearson and J.A. Thomson</i>	52
Thyroid antibodies in Africans with diffuse toxic goitre: Response to medical therapy <i>W.J. Kalk and J.C. Santos</i>	55
Heterogeneity in juvenile thyrotoxicosis <i>P.A. Dahlberg, P. Wilton and A.F. Karlsson</i>	58
Follow up of 35 Graves' patients thyroid stimulating antibody activity during treatment: A prospective study <i>G. Bakó and A. Leövey</i>	61
Monitoring of immunopathological events in Graves' patients during medical treatment: Immunological studies <i>U. di Mario, M. Vitillo, F.P. Cavatorta, A. Scardellato, F. Dotta, J. Sutherland, P. Pozzilli and D. Andreani</i>	64
Evolution of thyrotropin-receptor antibodies and microsomal antibodies after radioiodine treatment of Graves' disease <i>H.M. Heshmati, M. Izembart, F. Dagousset and G. Vallee</i>	67
Dithiothreitol inhibits thyroid cell stimulation of iodine organification by Graves' immunoglobulins <i>J. Ginsberg, D.J. Rafter and P.G. Murray</i>	69
 AUTOIMMUNE ASPECTS OF HYPOTHYROIDISM	
Autoimmune hypothyroidism <i>A. Pinchera, G. Fenzi, L. Chiovato and P. Vitti</i>	73
Adsorption of thyroid microsomal fraction on plastic materials <i>M. Gembicki, K. Łacka, J. Kosowicz and J. Seidel</i>	81
Evidence that thyroid peroxidase is the 'microsomal' antigen <i>B. Czarnocka, J. Ruf, M. Ferrand, S. Lissitzky and P. Carayon</i>	84
Evidence for identity of serum thyroid microsomal and anti-human thyroid peroxidase autoantibodies <i>S. Mariotti, R. Bechi, S. Anelli, J. Ruf, A. Lombardi, L. Chiovato, B. Czarnocka, P. Carayon and A. Pinchera</i>	86
Ultrastructural localization of microsomal antibodies on the surface of human thyroid follicle cells <i>M. Nilsson, J. Mölne, F.A. Karlsson and L.E. Ericson</i>	88

Interaction between GM ₃ ganglioside and immunoglobulins from mothers of hypothyroid infants <i>J.H. Dussault, D. Bernier and J. Ruel</i>	90
Antibodies against 'second colloid antigen' in sporadic congenital hypothyroidism <i>R.D. van der Gaag, A.M. Bloot and H.A. Drexhage</i>	93
Permanent and transient newborn hypothyroidism due to maternal autoimmune thyroiditis <i>M. Klett, U. Heinrich, M. Müller and D. Schönberg</i>	96
Hypothyroidism and autoimmune thyroid destruction: A role for thyroid autoantibodies? <i>S. McLachlan, R. Jansson, P. Whitehead, C. Pegg and B. Rees Smith</i>	99
Thyroid pathology and function in experimental autoimmune thyroiditis of inbred mice <i>S.C. Imahori and A.O. Vladutiu</i>	106
In vivo activated T cells with natural killer (NK) activity in the thyroid infiltrate of patients with Hashimoto's thyroiditis <i>G.F. Del Prete, A. Tiri, S. Mariotti, A. Pinchera, S. Romagnani and M. Ricci</i>	109
Activated HLA-DR- and IFN- γ -expressing cytotoxic/suppressor T cells in thyroid tissue and peripheral blood of patients with subacute thyroiditis <i>F.A. Karlsson, T.H. Tötterman and R. Jansson</i>	111
The effect of replacement therapy on thyroid antibody and serum thyrotropin concentrations in Hashimoto's thyroiditis <i>B. Trbojević, N. Lalić and D. Slijepčević</i>	113
Thyroid microsomal antibodies do not react with calcitonin-producing thyroid cells <i>W.A. Scherbaum, H. Mogel and E.F. Pfeiffer</i>	115
Thyroid autoimmunity in thyroid cancer <i>F. Pacini, S. Mariotti, N. Formica, S. Anelli, R. Bechi, R. Elisei and A. Pinchera</i>	117
Hyperthyroidism associated with histological Hashimoto's thyroiditis and thyroid papillary carcinoma: Persistent antibody titres during one year after total thyroid resection without metastases <i>M. Gomez-Balaguer, E. Caballero, P. Costa, A. Gilsanz, A. Ribas, E. Bernat and J. Esteban</i>	119
Immune dysregulation and the follow-up of athyrotic patients <i>M. Izembar, H.M. Heshmati, H. Nasser and G. Vallee</i>	121

- An investigation of thyroid hormone autoantibodies in thyroid disease
P. Merlin, A. Balsamo, L. Mongardi and V. de Filippis 123

ANTIGEN PRESENTATION AND THYROID AUTOIMMUNITY

- Inappropriate HLA class II expression of epithelial cells: Consolidation and progress**
I. Todd, R. Pujol-Borrell, M. Londei, M. Feldman and G.F. Bottazzo 127
- The role of DR-expression on thyrocytes for the autoimmunity of the thyroid
J. Teuber, R. Paschke, U. Schwedes and K.H. Usadel 139
- 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 and P.C. Scriba 141
- Role of methimazole on DR antigen expression on human thyroid epithelial cell cultures
J.-C. Carel, J.-J. Remy, D. Zucman, J. Salamero and J. Charreire 145
- Purification of a 20 KD peptidic fragment from thyroglobulin (Tg) responsible for immunoreactivity towards monoclonal anti-Tg antibodies blocking the primary syngeneic sensitization
J. Salamero, J.-J. Remy and J. Charreire 148
- Are conserved regions on thyroglobulin thyroiditogenic?
Y.M. Kong, A.A. Giraldo, J.M. Justen, L.L. Simon and B.E. Fuller 151
- Dendritic cells: Contributions to cell-mediated immunity**
R.M. Steinman 153
- Human thyroglobulin treatment of blood dendritic cells induces IgG antibody production in cultures from Hashimoto patients
J. Farrant, A.E. Bryant, J. Chan and R.L. Himsworth 164
- Antigen-presenting cells (APC's) and the thyroid auto-immune response in the BB/W rat
H.A.M. Voorbij, P.J. Kabel and H.A. Drexhage 166

AUTOIMMUNE ASPECTS OF SPORADIC AND ENDEMIC GOITER

- Thyroid growth stimulating immunoglobulins (TGI) and goitre**
H.A. Drexhage and R.D. van der Gaag 173
- Increased T helper/T suppressor-cytotoxic lymphocyte ratio in sporadic nontoxic goitre
A. Berghout, W.M. Wiersinga and H.A. Drexhage 187
- What is thyroid growth promoting activity ('TGI')?
R. Gärtner, W. Greil, C. Tzavella, A. Witte and C.R. Pickardt 191

Thyroid growth stimulation by a (FAB) ₂ -fragment prepared from an IgG of a TGI-positive nontoxic goitre patient <i>R.A.P. van der Meer, R.D. van der Gaag and H.A. Drexhage</i>	193
Possible significance of IgG thyroid stimulators in nontoxic goitre <i>P.P.A. Smyth, B. Grubeck Loebenstein and T.J. McKenna</i>	196
Thyroid growth promoting antibody in patients with non toxic goiter <i>C.M. Rotella, C. Mavilia, L.D. Kohn and R. Toccafondi</i>	198
Autoimmunity in nodular goiter <i>K. Westermark, P. Persson, H. Johansson and A. Karlsson</i>	202
Autoimmunity in black African multinodular goitres <i>J.E.C. Santos and W.J. Kalk</i>	204
Growth promoting effects of TSH and EGF in isolated porcine thyroid follicles and occurrence of thyroid growth stimulating immunoglobulins in euthyroid goitre patients from an iodine-deficient endemic goitre area <i>H. Schatz, R. Bär, F. Müller, J.-A. Nickel and H. Stracke</i>	207
Thyroid growth promoting activity in endemic goiter <i>A. Halpern, G. Medeiros-Neto and L.D. Kohn</i>	209
Thyroid autoantibodies after iodine supplementation <i>D.A. Koutras, K.S. Karaiskos, K. Evangelopoulou, M.A. Boukis, G.D. Piperigos, J. Kitsopanides, D. Makriyannis, J. Mantzos, J. Sfontouris and A. Souvatzoglou</i>	211
Thyroiditis induced by dietary iodine may be due to the increased immunogenicity of highly iodinated thyroglobulin <i>R.S. Sundick, D. Herdegen, T.R. Brown and N. Bagchi</i>	213
Amiodarone thyroid autoimmunity relationship? <i>M. Gomez-Balaguer, E. Caballero, P. Costa, A. Gilsanz, E. Bernat, C. Uriel and V. Alfonso</i>	215
Autoimmunity in nodular goiter? <i>H. Studer, H. Gerber and H.J. Peter</i>	217
Studies on the natural history of toxic nodular goitre <i>P.P.A. Smyth, D.F. Smith and T.J. McKenna</i>	227
Intrathyroidal helper/inducer T cells and activated T cells in patients with euthyroid nodular goiter <i>A. Balsamo, F. Botto Micca, P. Merlin, V. de Filippis and A. Stramignoni</i>	229
Intrathyroidal immunological abnormalities in variants of endemic goitre <i>B. Grubeck-Loebenstein, K. Krisch, H. Kassal, P.P.A. Smyth and W. Waldhausl</i>	231

Morphometric evidence for regional differences of thyroid hyperfunction in Graves' disease caused by lymphocytes <i>R. Paschke, P. Pfiester, A. Hessenbruch, J. Winter, J. Teuber, U. Schwedes and K.H. Usadel</i>	234
Structural and functional heterogeneity in Graves' disease tissue <i>H.-W. Müller-Gärtner and C. Schneider</i>	236
AUTOIMMUNE ASPECTS OF ENDOCRINE OPHTHALMOPATHY	
Humoral immunity and endocrine ophthalmopathy <i>P. Kendall-Taylor</i>	241
Thyroid antibodies in endocrine ophthalmopathy <i>K. Bech, U. Feldt-Rasmussen, Å. Krogh Rasmussen, M. Blichert-Toft, H. Bliddal, S. Faurschou and D. Marushak</i>	245
Humoral immunity in Graves' ophthalmopathy <i>J. Nauman, M. Faryna and A. Gardas</i>	248
Detection of IgG and IgM antibodies directed against eye and peripheral muscle preparations in juvenile and adult patients with Graves' disease and in patients with hypothyroidism <i>U. Bemetz, R. Moncayo, W. Scherbaum, H. Frisch, K. Badenhoop, G. Hool, H. Schleussener and E.F. Pfeiffer</i>	250
Spontaneous and therapy induced course of eye muscle antibodies in endocrine ophthalmopathy (EO) <i>G. Kahaly, R. Moncayo, U. Bemetz, U. Krause, J. Schrezenmeir, J. Beyer and E.F. Pfeiffer</i>	253
Current status of autoantibodies against orbital antigens in the pathogenesis of Graves' ophthalmopathy <i>M. Salvi, Y. Hiromatsu, L. Wosu, E. Laryea, J. How and J.R. Wall</i>	255
Preliminary characterization of the soluble eye muscle antigen in the 80000xg supernatant using FPLC-anion exchange chromatography <i>R. Moncayo, U. Bemetz and E.F. Pfeiffer</i>	267
Studies of cell-mediated immunity in Graves' ophthalmopathy using the antigen-induced leukocyte procoagulant activity assay <i>J. How, A. Miller, E.A. Laryea and J.R. Wall</i>	270
Role of HLA class II antigen expression on normal human eye muscle cells and orbital fibroblasts in Graves' ophthalmopathy <i>Y. Hiromatsu, L. Wosu, J. Dornan and J.R. Wall</i>	272
Lymphocyte subsets in Graves' ophthalmopathy patients <i>R. van der Gaag, L. Broersma, L. Koornneef and W.M. Wiersinga</i>	275

Treatment of severe Graves' ophthalmopathy with cyclosporin A alone and in combination with corticosteroids <i>Ch. Utech, K.G. Wulle, E. Bieler and N. Panitz</i>	278
IMMUNE DYSREGULATION IN THYROID DISEASE	
The role of immune dysregulation in the pathogenesis of autoimmune thyroid disease <i>R. Volpé</i>	283
Activated (Ia ⁺) T-cell subsets in autoimmune thyroid diseases <i>J.Y.C. Chan and P.G. Walfish</i>	294
Circulating activated T cells in Graves' disease <i>N. Genetet, G. Merdrignac, H. Allannic, G. Edan, A. Roughol and B. Genetet</i>	296
Impact of T helper and T suppressor cells on the production of anti-thyroglobulin antibodies in autoimmune thyroiditis <i>J. Petersen and U. Feldt-Rasmussen</i>	299
In vitro production of thyroglobulin specific helper factor in humans <i>K. Sato, Y. Hara, S. Sakurai, Y. Sakatsume, T. Tanikawa, J. Ishii and L.J. Degroot</i>	302
Is cellular immunity to the TSH receptor restricted to patients with Graves' disease? <i>S. Vento, C.J. O'Brien, T. Cundy, R. Williams and A.L.W.F. Eddleston</i>	304
B cell activity in Graves' disease <i>R. Wilson, J.H. McKillop and J.A. Thomson</i>	306
Antigen and mitogen induced differentiation of circulating B cells in patients with autoimmune thyroid disease (AIT) and normal individuals <i>T. Logtenberg, A. Kroon, F.H.J. Gmelig-Meyling and R.E. Ballieux</i>	308
Thyroglobulin epitopes and anti-thyroglobulin idiotypes in autoimmune thyroiditis <i>D. Male, G. Pryce, B. Champion and I. Roitt</i>	311
Detection and significance of anti-idiotypic-AB(a-ID-Ab) for autoimmune thyroid diseases <i>U. Schwedes, J. Teuber, R. Paschke, J. Christophel and K.H. Usadel</i>	319
Modulation of the immune response by thioureyline anti-thyroid drugs <i>D.J. Kerr, M.M. Ferguson, J. McGroarty, A.R. McLellan and W.D. Alexander</i>	321
Serum thyrotrophin receptor antibody (TRAb), thyroxine (T ₄) and Tri-iodothyronine (T ₃) levels in patients receiving carbimazole and propylthiouracil (PTU) <i>J.A. Thomson, R. Wilson and J.H. McKillop</i>	323

Graves' disease patients treated with carbimazole: Thyroid stimulating immunoglobulins (TSab) assay and T cell subset counts after 5 years of remission following drug withdrawal <i>H. Allanic, N. Genetet, N. Massart and B. Genetet</i>	326
Index of authors	329
Subject index	333

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

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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 10⁴/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 β -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 1:320²; 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.

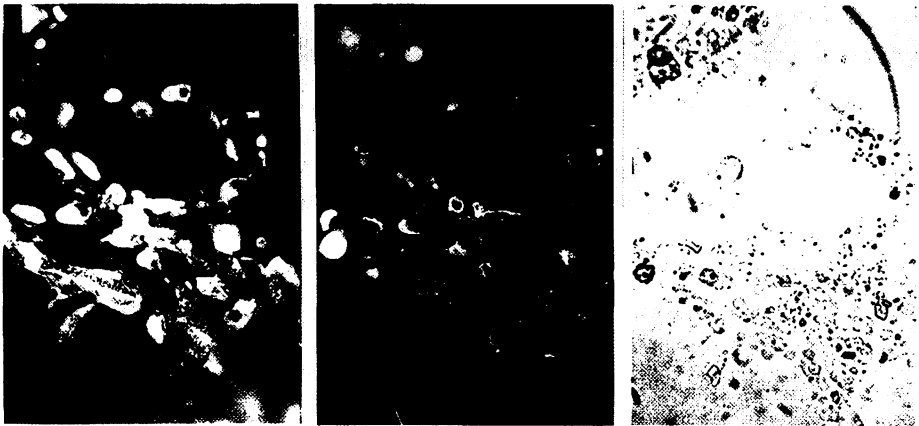


Fig. 1. HLA-DR and M-antigen expressed by thyroid monolayers preincubated with 10 mU/ml bTSH in 5H medium; left: bright light; middle: HLA-DR; right: M-antigen. Original magnification $\times 250$

RESULTS

We demonstrated simultaneous 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.

	SH Medium			FCS			n
	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
 * - hyperthyroid GD-patient
 NTG - non toxic nodular goiter

b - expressed spontaneously
 o - euthyroid GD-patient
 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 IFN γ with cyclosporin A gave inconclusive results. Incubation of TMs with 5 μ g/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 spontaneously (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, although an induction of class II antigen via IFN would lead preferentially to -DQ expression of TMs(7)

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