Zajímavé kasuistiky/nálezy celiakie?

Ivo Lochman, Vítězslav Novák,
Alexandra Lochmanová, Alena Kloudová
odd. imunologie a alergologie ZÚ Ostrava

XV. ČASOMIL, Rychnov nad Kněžnou, 1.-3.6.2011

Atypické případy celiakie

MUDr. Vítězslav Novák
oddělení imunologie a alergologie
Zdravotní ústav se sídlem v Ostravě

MDI, Martin, 2009

před GFD

4 měsíce po zahájení GFD

Může být vůbec nějaká souvislost mezi dacryoadenitidou a celiakií ?

S.M., žena, nar. 1973

Dg: 2008-dacryoadenitis l. dx.
popisný název (zánět slzné žlázy)
histologie: reaktivní lymfoidní hyperplazie
negativní byl rozsáhlý panel autoprotilátek i infekční sérologie, špatná byla reakce na systémové kortikoidy
Z rozpaků uzavíráno jako“primární“

2008

EMA IgA pozitivní
anti-tTG IgA silně pozitivní, IP 10,6
anti-DGP IgA pozitivní, IP 2,1
anti-GL IgA pozitivní, IP 2,1
Prokázána riziková alela DQ2
Biopticky z D2 duodena potvrzena celiakie

po 4 měsících GFD

EMA IgA pozitivní
anti-tTG IgA pozitivní, IP 2,21
anti-DGP IgA negativní IP 0,72

Dnes všechny sérologické markery celiakie negativní, znatelné klinické zlepšení

Celiac Disease – Do we understand it?

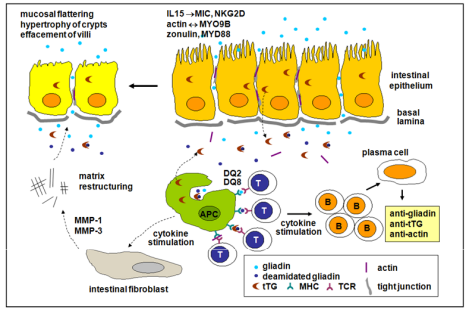
Lochman I., Novák V., Lochmanová A.
Department of Immunology and Allergy, Institute of Public Health, Ostrava, Czech Republic

Case report

Backgrounds and Aims

Celiac disease (CD) is an immune-mediated enteropathy caused by sensitivity to wheat gluten in genetically susceptible individuals [1]. Clinical manifestations range from typical gastrointestinal problems and failure to thrive in children, to a diverse range of symptoms in adults. The most prominent pathological change is flattened villae (fig.1) observed in biopsy of the small intestine. The causes of this pathophysiologic change are complex. All symptoms and pathology disappear with a gluten-free diet (GFD) that usually needs to be kept lifelong. Besides biopsy, the laboratory diagnosis and monitoring of CD is based on the determination of IgA anti-gliadin antibodies that induces the disease and anti-tissue transglutaminase (tTG) antibodies by ELISA or detection of anti-endomysial antibodies (EMA) by indirect immunofluorescence (IIF). EMA has the same diagnostic value as the determination of anti-tTG; because tTG is the antigen in the endomysium. IgA deficiency has to be always excluded when negative results of IgA anti-gliadin and anti-tTG or EMA are found.
Common anti-gliadin ELISAs are not very sensitive or specific compared to anti-tTG and EMA. It was proved that very specific and sensitive for celiac disease are antibodies against deamidated gliadin peptide (anti-DGP) and that the ELISA against these antibodies are comparably sensitive and specific with anti-tTG and EMA.
The aim of this study was to present the case of the patient that was suffered from celiac disease in the adult age, was improved after GFD but the CD was not appear when the GFD was terminated.

Fig. 1: Pathophysiology of celiac disease



Gliadin in food as well as infectious attacks can induce zonulin release from intestinal epithelial cells. Activation of the zonulin pathway by PKC mediates cytoskeleton reorganization due to actin polymerization and tight junction opening leads to a rapid increase in intestinal permeability to macromolecules irrespective of the expression of autoimmunity. Some variations in human myosin IXB (MYO9B), which has a role in actin remodeling of epithelial enterocytes, can lead also to impairment of the intestinal barrier in the etiology of celiac disease. Anti-enterocyte antibodies found in patients suffering from intestinal villous atrophy are directed against actin filaments and are associated in CD patients with the anti-tTG positivity. Some gliadin peptides, like p31-43, delay inactivation of epidermal growth factor receptor (EGFR) through interference with endocytic pathway and thus amplify the effects of trace amounts of EGF on actin cytoskeleton. They also lead to overexpression of MIC molecules on mucosa and production and release of IL-15 which induces expression of its ligand NKG2D on intraepithelial lymphocytes (IEL). Interaction of NKG2D and MICA tends to destruction of mucosa epithelial cells. Transglutaminases (TGs) are ubiquitous enzymes with many functions. There are several forms of TGs with high homology. Each of them is localized in different compartments of different tissues. The main form of small intestine TG is tTG (TG2). Inflammatory process induced by increased amount of gliadin in submucosa leads to the release of tTG from participating cells and amplification of destructive mechanisms mediated by cytokines, with metalloproteinases leading to mucosa transformation. Gliadin is deamidated in intestinal submucosa by tTG, which cross links gliadin. We must remember that gliadin is not normal physiologic substrate for tTG.

Patient report

We report the case of 35 years old man suffered only from pollinosis beginning in the childhood (spring pollen, grasses + rye confirmed by sp.IgE positivity). No thyropathy or autoimmunity was recorded not even in relatives in their history. He was investigated for fatigue syndrome in 2001in our department in the first time. Typical ultrasonic picture of chronic autoimmune thyroiditis with weak anti-TPO and anti-TG positivity was found. Antibodies against parietal cells and also against F-actin in IgG class were detected. Hypothyroidism was treated by hormonal substitution. Antibodies against tTG and DGP in IgA class were found about a year later, antibodies against F-actin in IgA class were negative. Antibodies against DGP in IgG class were negative. HLA-DQA1 0501/DQB1 0201 was positive. Endoscopic examination found atrophic gastritis with well preserved but weak acidity. Smoothed relief was observed in duodenum with increased number of CD3+ intraepithelial lymphocytes (80/100). Jejunum histology corresponded with stadium IIIb of modified Marsh classification. Our conclusion for patient status that time: typical case of celiac disease (CD).

Two years after strict gluten-free diet (GFD) patients became negative for anti-tTG and DGP. All clinical symptoms have disappeared. Patient has started to add gluten to his diet gradually. All laboratory indicators of CD have remained negative up to now as well as endoscopic and histologic jejunal findings. Patient informs us that he is suffered from no health problems except pollinosis nowadays. He remains positive in IgG anti-F-actin antibodies and anti-striated muscle antibodies.

Applied laboratory methods

All mentioned laboratory examinations were performed by routine laboratory methods applied in the laboratories of Department of Immunology and Allergy of Institute of Public Health in Ostrava (http://www.zuova.cz/home/laboratorni_prirucka_cld.pdf). Anti-tTG and anti-DGP antibodies in IgA class were determined using ALBIA (Quanta-Plex Celiac IgA Profile, Inova Diagnostics, Inc.), anti-tTG, anti-DGP and anti-F-actin antibodies were determined using Quanta-Lite ELISAs (Inova Diagnostics, Inc.). Anti-TPO and anti-TG antibodies were determined using ALBIA (Fidis-Thyro, BMD) and anti-TSHr using Anti-TSH Receptor ELISA (IgG), Euroimmun.

Results

Tab. 1: Selected laboratory results of patient M.T. , man, born in 197x

date	anti-DGP	anti-tTG	anti-DGP	anti-tTG	anti-endo	anti-F-actin		anti-TPO	anti-TG	anti-TSHr	anti-StMA	anti-PCA	anti-IF
	IgA		IgG		IgA	IgA	IgG	IgG					
23.12.2003	4,95	2,15	0,20	1,803				0,52	1,467	1,497	positive	negative	negative
17.10.2006	0,28	0,24	0,02		negative	0,43	3,3	2,251	1,317	1,781	positive	weak pos	negative
25.1.2010	0,28	0,23	0,16			0,46	5,8	0,898	1,715	0,868	weak pos	weak pos	negative

StMA - striatated muscle, PCA – parietal cell antibody, IF - intrinsic factor, DGP – deamidated gliadin peptide, tTG – tissue transglutaminase, endo – endomysium, TPO – thyroperoxidase, TG – thyroglobulin, TSHr – thyroid stimulation hormone receptor . Results are given in positivity indexes (PI = patient's value/cut-off value).

Discussion

Attention should be paid to the following facts and presumptions when talking about CD:

- CD is a complex disease induced by gliadin in genetically predisposed patients.
- The autoantigen in CD is tTG (TG2) that participate on gliadin metabolism inside the intestinum.
- Transglutaminases (TG) are enzymes with high homology. Once released, antibodies against particular TG isotype can react also with other TG isotypes present in other tissues than intestinum.
- Tissue transglutaminases (tTG =TG2, EC 2.3.2.13) are ubiquitous enzymes that catalyze posttranslant Ca^{2+} dependent modification of proteins by highly stabile (ε-[γ-glutamyl]) lyzine isopeptide bonds insertion or by polyamine connections to certain peptide bound glutamine residues. $TG2^{c}$ show also GTP-ase activity and can be effective also as G-proteins transmitting signal to some hormones.
- Deamidated gliadin epitopes (DGP) are important in CD pathogenesis. They are released during interaction of gliadin with tTG. DGPs have up to 400-fold higher avidity do HLA DQ antigens then native gliadin peptides.
- Gliadin is not specific substrate for tTG [fig. 1].
- Anti-tTG antibodies from CD patients suffered from CD stimulate its transamidating/deamidating activity more then twice but reduce its GTP-ase activity as to 50%.
- tTG takes an active part in coagulation (supports fibronectin-integrin binding) and apoptosis.
- Anti-TG antibodies are found also in many inflammation, neurological and malignant diseases. Such diseases can be successfully affected with GFD.
- Serology is very effective tool for CD diagnosis and monitoring in the present. It is more reliable to test several different antibody specificities than doing two technologies on the same antigen, for instance like anti-tTG ELISA and EMA IIF, which are both measuring anti-tTG. Optimal composition of serological tests for CD, based on current knowledge of pathogenesis of this disease, might be in the future:
  - **Determination of zonulin level**: Increased zonulin levels lead to increased intestinal permeability to macromolecules including gliadin. But testing is not widely available outside the research laboratories in the present.
  - **Determination of anti-deamidated gliadin peptide antibodies level**: IgA antibodies against deamidated gliadin peptides are preferred over regular anti-gliadin because the former are both more sensitive and specific. Gliadin is the agent that incites the clinical symptoms of the disease. It is tempting to speculate that gliadin that has been deamidated *in vivo* is the main antigen that starts the immune response in CD, and tTG crosslinked to deamidated gliadin becomes immunogenic at the site of inflammation.
  - **Determination of anti-tTG or anti-endomysial antibodies**: Testing is widely available. Correctly performed, EMA and anti-tTG have the same diagnostic value. The antibodies in IgA class are of higher diagnostic value than the antibodies in the IgG class for the individuals without IgA deficiency. This is true in typical CD when intestinal enteropathy and gastrointestinal discomfort are the main symptoms. Antibodies in the IgG class can have the same or higher diagnostic value than IgA antibodies for patients suffered from atypical symptoms (dermatological, neurological etc.).
  - **Determination of anti-F-actin antibodies in IgA class**: Anti-F-actin levels may yield an indication of the actual rate of destruction of intestinal mucosa enterocytes due to the pathophysiological process induced by gliadin. But anti-actin antibodies are not specific for celiac disease. Tests have limited accessibility.
- Histological examinations of intestine biopsy samples using Marsh criteria remains the important tool for information about the intestine damage degree. But it has to be remembered that described changes are not CD specific and that this examination is invasive and patient inconvenient.

Conclusions

Regarding presumptions given in the discussion, we afford to conclude:

- **CD is the complex disease which comes out in susceptible individuals when gluten is the part of diet.**
- **CD is induced due to intestine permeability failure and increased gluten input through the intestine epithelium into the intestine lumen in some individuals. In such individuals not lifelong GFD is necessary to treat successfully this disease when the intestine permeability failure is removed.**

References

1. Lochman I., Martis P., Burlingame R.E., Lochmanová A.: Multiplex Assays to Diagnose Celiac Disease. Ann.N.Y.Acad.Sci. 2007, 1109; 330-337
2. Facchiano, F. *et al*. 2006. The Role of Transglutaminase-2 and its substrates in Human Diseases. Frontiers in Bioscience 11:1758-1773
3. Mehta, K. *et al*. 2006. Tissue Transglutaminase: From Biological Glue to Cell Survival Cues. Frontiers in Bioscience 11:163-185
4. Aleadin A., Green P.H.R.: Narrative Review: Celiac Disease: Understanding a Complex Autoimmune Disorder. Ann Intern Med. 2005;0 142: 289-298

MD
7th
23

M.T., muž, nar. 1976

polinotik od dětství

2001

vyšetřován pro únavový syndrom, **bez gastrointestinálních potíží**
diagnostikována chronická thyreoitída (imunogenní struma s anti-TPO a anti-TG pozitivitou, hypotyreózou)

2002

provedeno také vyšetření na celiakii: anti-tTG, anti-DGP a anti-F-aktin ve třídě IgA pozitivní, heterodimer HLA-DQA1 0501/DQB1 0201 pozitivní, alela DRB1 04 negativní, endoskopicky i histologicky obraz **klasické celiakie**
Nasazena GFD do roka negativizace laboratorních markerů celiakie

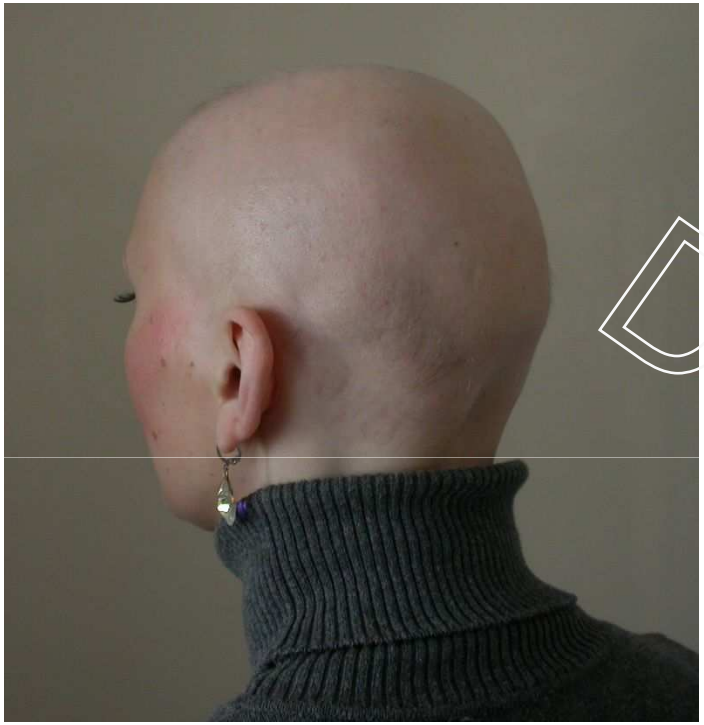
2004

kontrola, muž již cca rok nedodržuje GFD, bez potíží, anti-tTG, anti-DGP a anti-F-aktin ve třídě IgA negativní,Endoskopie a histologie negativní ve smyslu celiakie

2011

Je skutečně nutné u všech případů celiakie dodržovat naprosto striktní celoživotní bezlepkovou dietu?

H.U., žena, nar. 1982



Vyšetřována pro ložiskovou alopecii s vývojem alopecie totální **bez jakékoliv gastrointerstinální symptomatologie** Žádná endokrinopatie nebyla prokázána, laboratorní markery atopie byly negativní

2010

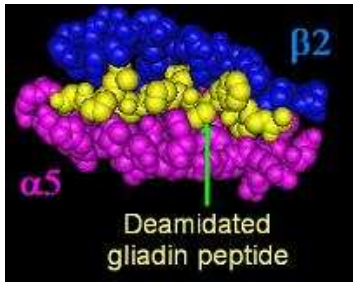
EMA IgA pozitivní
anti-tTG IgA silně pozitivní, IgG sl.pozit.
anti-DGP IgA hraniční, IgG hraniční
anti-GL IgA negativní, IgG negativní
rizikové alely DQ2 a DQ8 negativní

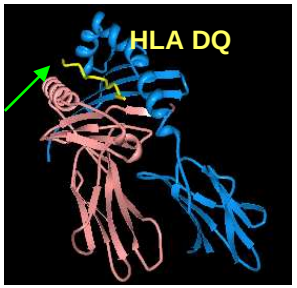
Endoskopie : Erytém sliznice duodena a vyhlazené řasy,nález odpovídá malabsorbčnímu syndromu

Histologie : Prosáklé fragmenty tenkého střeva s obrazem totální atrofie slizničního reliéfu resp. klků. IEL zmnoženy nad 40/100 enterocytů, hyperplazie krypt. **Histologie odpovídá celiakii ve stadiu Marsh IIIc**

Na zhodnocení efektu GFD je ještě příliš brzy

Je možné hovořit o celiakii, jestliže jsou anti-DGP a anti-GL negativní?
Je příčinná souvislost mezi celiakií a alopecií?





Rizikové alely u celiakie

alela	cis-haplotyp		haplotyp	
	izoforma	subtyp	DQA1	DQB1
HLA-DQ2	DQ $\alpha^5\beta^2$	DQ2.5	*0501	*0201
HLA-DQ8	DQ $\alpha^3\beta^8$	DQ8.1	*0301	*0302

V Evropě jen 6% celiaků nemá rizikové alely DQ2.5 nebo DQ8. Z nich 4% mají DQ2.2 izoformu a jen 2% nemají DQ2 nebo DQ8. Jen u 5% jedinců s rizikovou alelou HLA-DQ2 dojde k propuknutí celiakie, ale riziko je 250x vyšší než u jedinců, kteří ji nemají. (Wikipedia)

průkaz v krvi	sensitivita	specifičnost
HLA-DQ2	94%	73%
HLA-DQ8	12%	81%

Hadithi M. et l.: Ann.Intern.Med. 2007; 147(5): 294-302

J.G., muž, nar.1958

dg: torpidní refrakterní celiakie zjištěna před 15 lety (potvrzena histologicky 2x, ale podle tehdy uznávaných kriterií)
nereaguje na dietu
ani na léčbu systémovými kortikoidy

2005

EMA IgA negativní
anti-tTG IgA silně pozitivní

2011

EMA IgA negativní, IgG negativní
anti-tTG IgA pozitivní, IgG negativní
anti-DGP IgA negativní, IgG negativní
anti-GL IgA silně poz, IgG poz, IgE neg
rizikové alely: DQ2 neg, DQ8 pozitivní
IgG 6,0 g/l, IgA 21,6 g/l, IgM 0,25 g/l, IgD <22 U/L, IgE < 2 kIU/l, monoklon.Ig neprokázán,podtřídy IgA1,2 zvýšeny symetricky

Jak lze vysvětlit jasnou pozitivitu anti-tTG a negativitu EMA ve třídě IgA u tohoto muže?
Jde o vůbec o celiakii? (negativní anti-DGP)

Definice celiakie

- Celiakie je enteropatie způsobená zvýšenou vnímavostí na gluten u geneticky predisponovaných jedinců, na jejíž patogenezi se podílí imunitní systém.
- Může mít širokou klinickou manifestaci od typických gastrointestinálních potíží nalézaných především u dětí, až po neurologické, kožní (Morbus Düring – dermatitis herpetiformis) aj. potíže u dospělých.
- Nejtypičtějšími průvodními gastrointestinálními patologickými změnami je destrukce klků a zbytnění krypt na sliznici tenkého střeva.
- **Jediným spolehlivých diagnostickým kritériem celiakie je stále i dnes vymizení klinických příznaků po nasazení bezlepkové diety**

Fáze vzniku celiakie

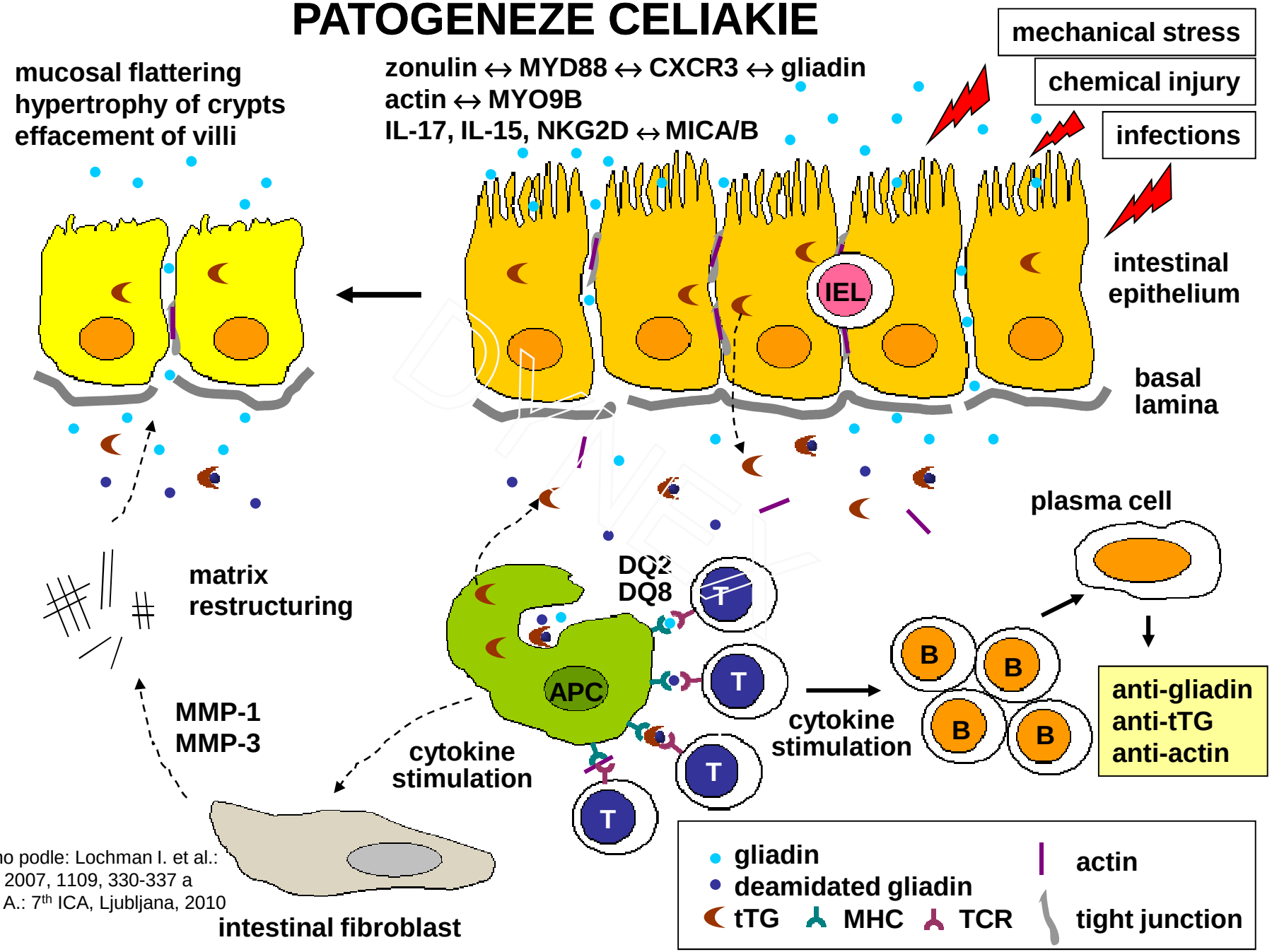
- Procesy probíhající ve střevním epitelu u zdravých, GS i CD jedinců – doba trvání: hodiny
- Reakce imunitního systému na bázi přirozené imunity u GS a CD jedinců - doba trvání: dny
- Reakce imunitního systému se zapojením adaptivní imunity u jedinců s CD - doba trvání: týdny - léta

A. Fasano, 7th International Congress on Autoimmunity, Ljubljana, 2010

Genetická predispozice k CD

- HLA-DQ2 a HLA-DQ8 jsou hlavními genetickými komponentami, které predisponují k CD
- Riziko vzniku CD odpovídá kombinaci HLA haplotypů
- Alely HLA-DQ2 a HLA-DQ8 jsou rozšířeny po celém světě
- Prevalence HLA-DQ2 v běžné populaci koreluje s prevalencí CD
- Přítomnost rizikových haplotypů DQ2 a DQ8 je pro vznik CD nezbytná, ale ne dostačující
- I u jednovaječných dvojčat normálně zatěžovaných lepkem ve stravě je shoda v incidenci CD max. 21-70% (u dvojvaječných jen 0-13%)

PATOGENEZE CELIAKIE



upraveno podle: Lochman I. et al.: ANYAS 2007, 1109, 330-337 a Fasano A.: 7th ICA, Ljubljana, 2010