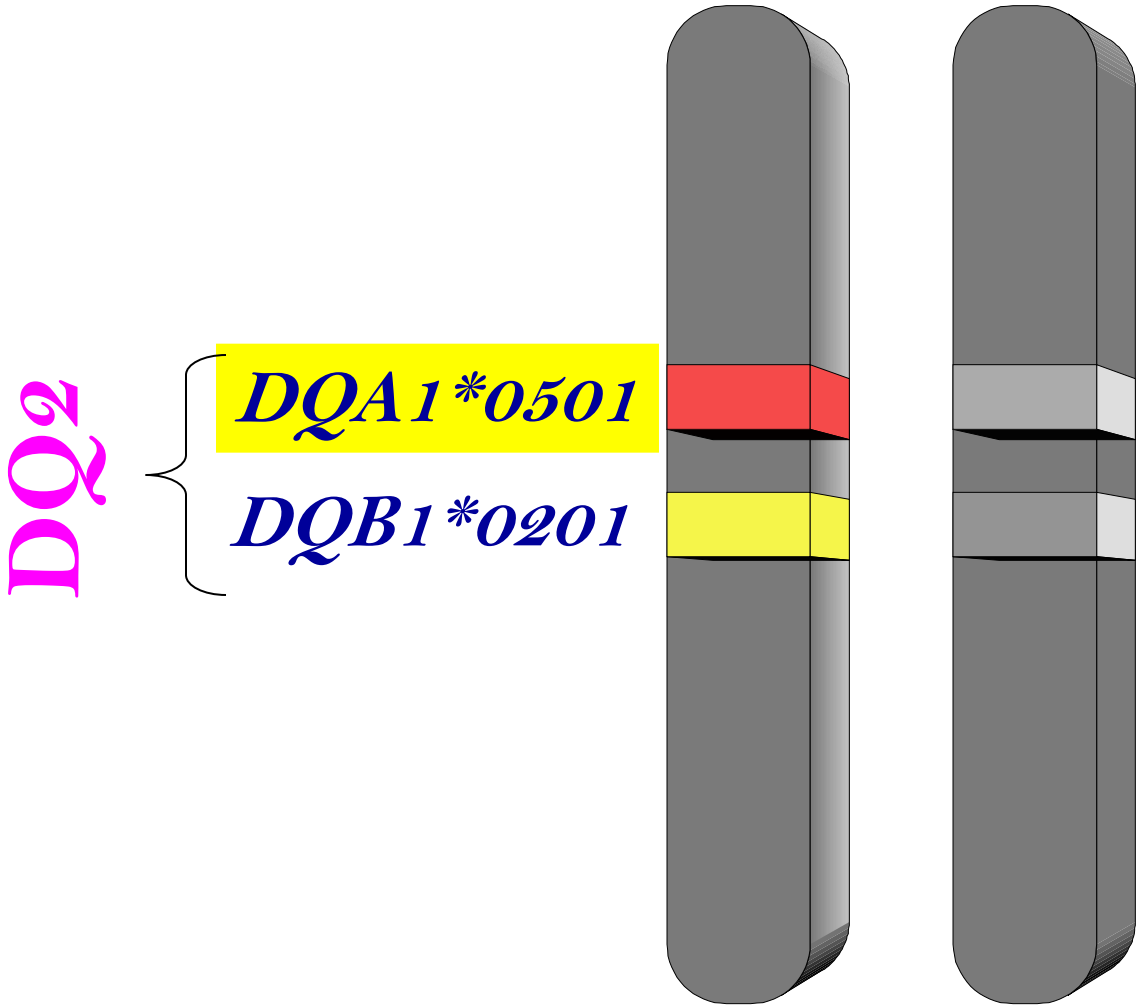
Background I

Celiac Disease (CD) is an intestinal disorder with multifactorial etiology. This chronic inflammatory disease is triggered by the ingestion of wheat gluten or related proteins from rye and barely in genetically-predisposed individuals. The disease is associated with specific HLA alleles indicating that HLA genes contribute to CD genetic susceptibility. Given the indisputable role of gluten in causing inflammation and immune-mediated tissue damage, CD represents a unique model of autoimmunity in which, in contrast to other autoimmune diseases, a close association with HLA genes (DQ2 and/or DQ8), a highly specific humoral autoimmune response (autoantibodies to tissue transglutaminase), and, most importantly, the triggering environmental factor (gluten), are known. The disease shows a strong human HLA association predominantly to HLA-DQ2 (A1*0501, B1*0201) and/or DQ8 (B1*0302) heterodimer (Fig. 1).

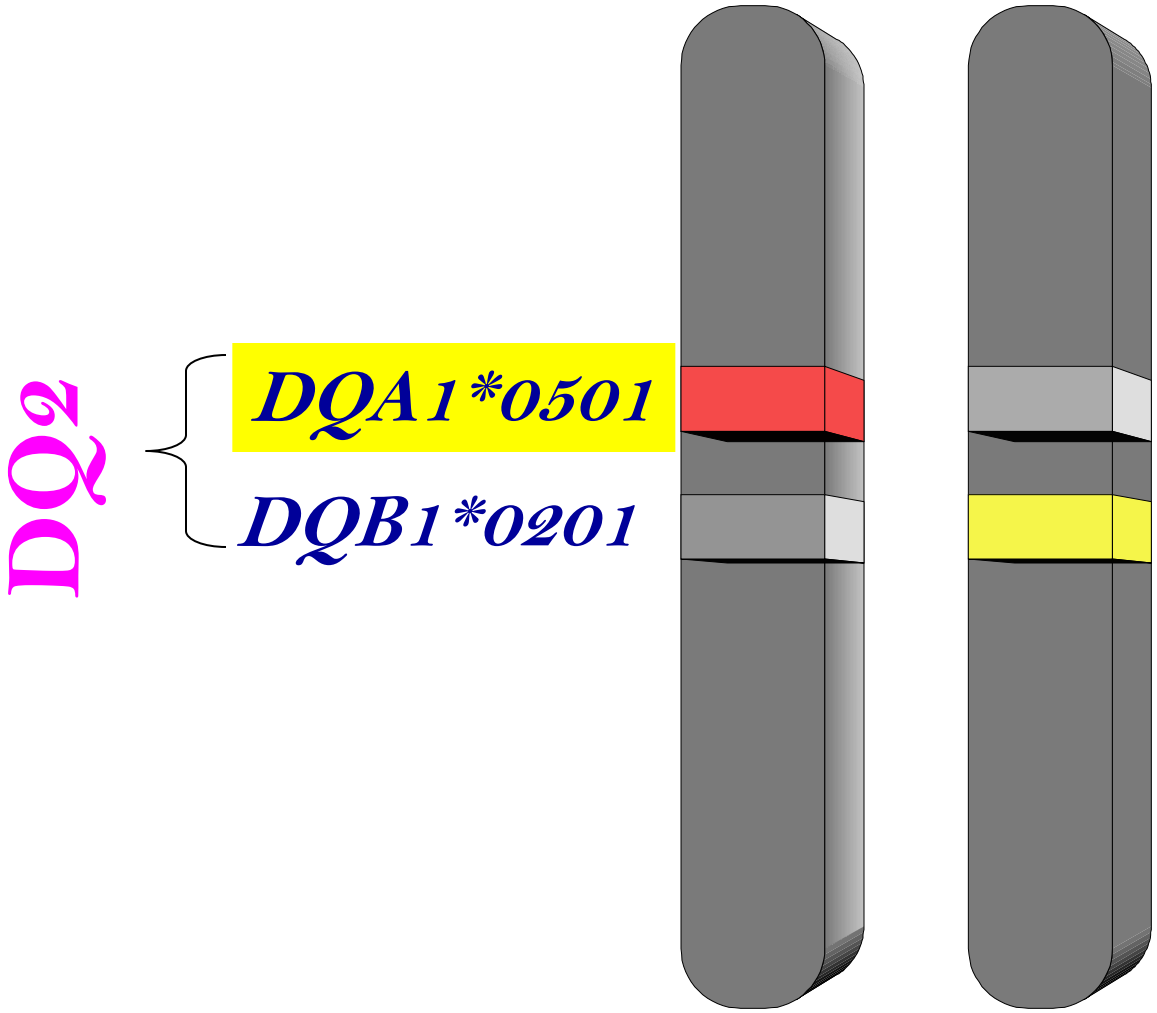
Linkage disequilibrium



CIS

Figure 1

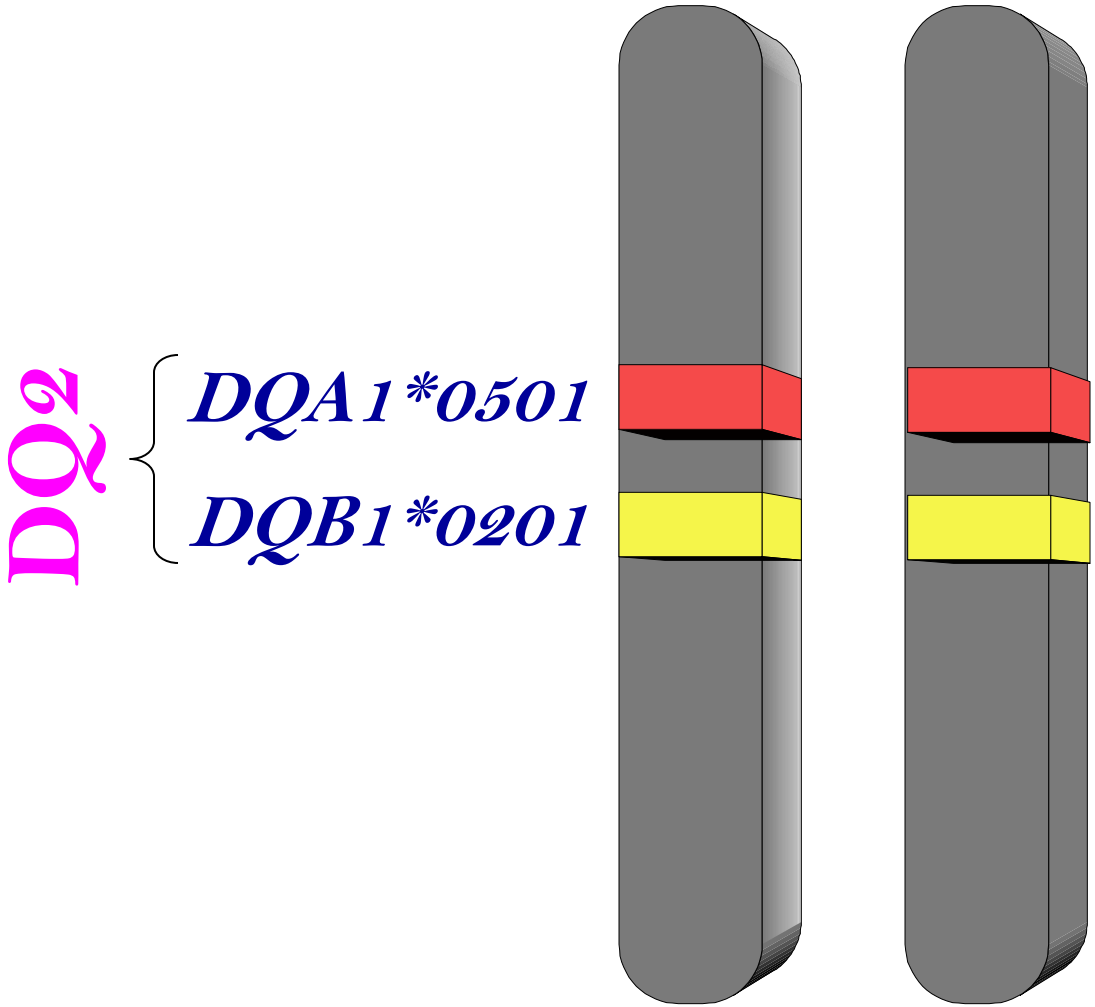
Linkage disequilibrium



TRANS

Figure 1

Linkage disequilibrium



CIS

Figure 1

Linkage disequilibrium

DQ8

DQA: Any

*DQB1*0302*

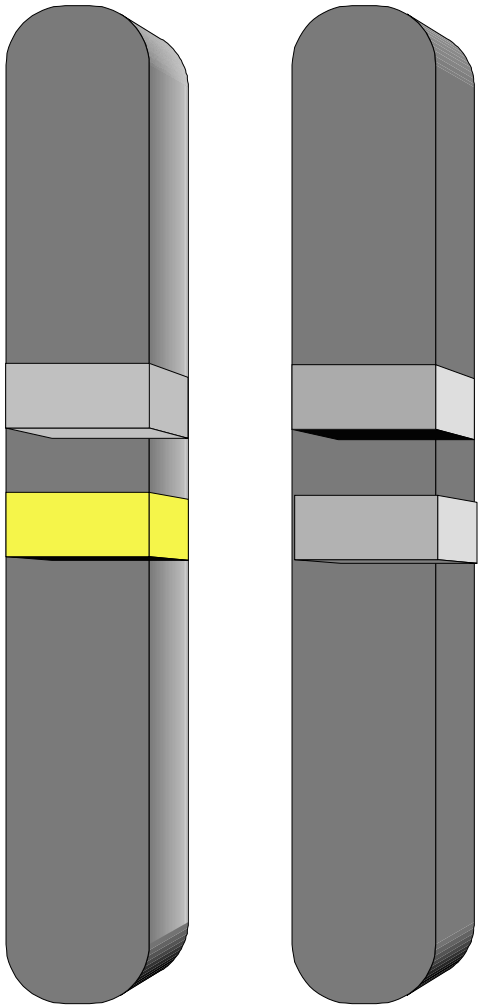


Figure 1

DQ2

A1*0501

B1*0201

DQ8

–

B1*0302

95% CD patients

5% DQ2/DQ8 negative????

Background II

The HLA class II genes play a key role in CD pathogenesis. HLA DQ2 and, less efficiently, DQ8 are the antigen presenting cells surface receptors for deamidated toxic gliadin fragments (Fig 2). Despite that their presence is considered necessary for CD pathogenesis, there is a low percentage of CD patients that are DQ2/DQ8 negative. However it has recently been reported that HLA DQ2 haplotype can be coded by the previously not described A1*0201 allele.

Antigen Presenting Cell and its Role in CD

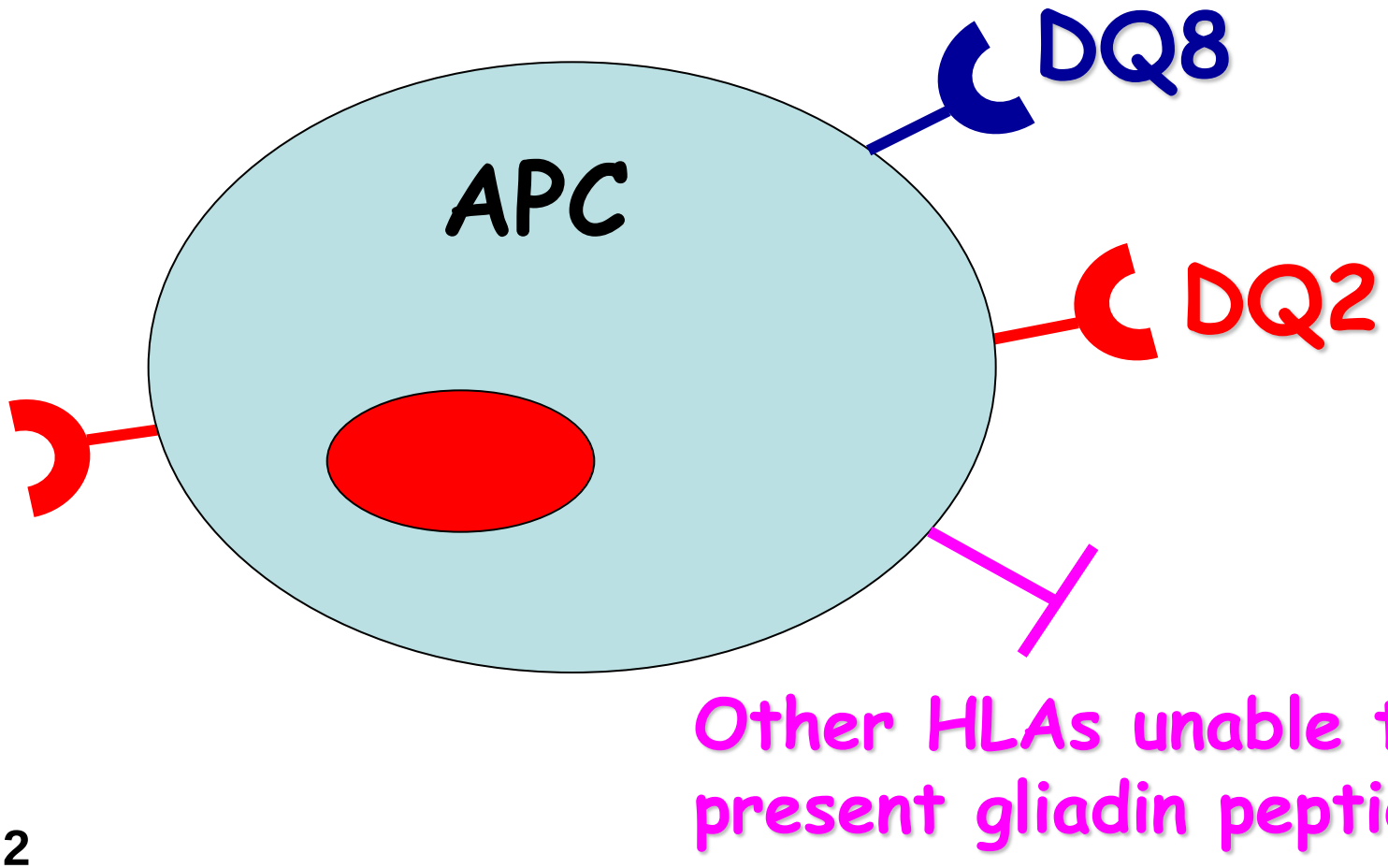


Figure 2

Aim

To verify whether patients classified as DQ2/DQ8 negative harbor the A1*0201 allele both in European and North American populations.

Methods

The HLA typing was performed in biopsy-proven CD patients.
Genomic DNA was extracted from whole blood samples; the DNA obtained was subjected to PCR using the Eu-DQ® Kit (Eurospital, Trieste - Italy). This Kit contains multiplex PCR reactions for DQA1*0501, DQA1*0201, DQ B1*0302, and DQ B1*0201 primers. Beta globin primers were used as internal control. The amplicons were resolved on 3% agarose gel and stained using ethidium bromide.

Results I

	U.S.A.				Europe			
	CD Patients		Controls		CD Patients		Controls	
Total (N)	78		40		100		60	
	N	%	N	%	N	%	N	%
DQ2 (A1*0501/B1*0201)	45	57.69	9	22.50	58	58.00	14	23.33
DQ2 (A1*0501+ A1*0201/B1*0201)	14	17.95	2	5.00	16	16.00	2	3.33
DQ2 (A1*0201/B1*0201)	6	7.69	2	5.00	5	5.00	3	5.00
DQ2/DQ8 (A1*0501/B1*0201 & B1*0302)	6	7.69	4	10.00	8	8.00	3	5.00
DQ2/DQ8 (A1*0201/B1*0201 & B1*0302)	0	0.00	2	5.00	1	1.00	1	1.67
DQ8 (B1*0302)	7	8.97	6	15.00	12	12.00	3	5.00
DQ2/DQ8 negative	0	0.00	15	37.50	0	0.00	34	56.67

Table 1

Results II

As reported in literature 95% of European CD patients tested positive for DQ2 and/or DQ8 using conventionally genotyping. The remaining 5% harbored the A1*0201/B1*0201 haplotype (see Table 1). Similarly in American CD patients 7.7% of DQ2/DQ8 negative subjects as established by the classical HLA genotyping also tested positive for A1*0201/B1*0201 allele. (see Table 1).

Therefore the A1*0201 allele accounted for the previously described DQ2/DQ8 negative CD patients, irrespective of geographical differences.

DQ2

A1*0501

B1*0201

DQ8

B1*0302

95% CD patients

A1*0201

B1*0201

5% CD patients
(previously negative)

Conclusions

Our data showed that A1*0201 allele accounts for all DQ2/DQ8 negative, biopsy-proven CD patients.

These results suggest that this allele should be included for the appropriate genomic screening of CD patients

The presence of DQ2 and/or DQ8 is confirmed to be crucial for deamidated gliadin presentation by antigen presenting cells as a key step in the pathogenesis of the intestinal damage typical of celiac disease (figure 3).

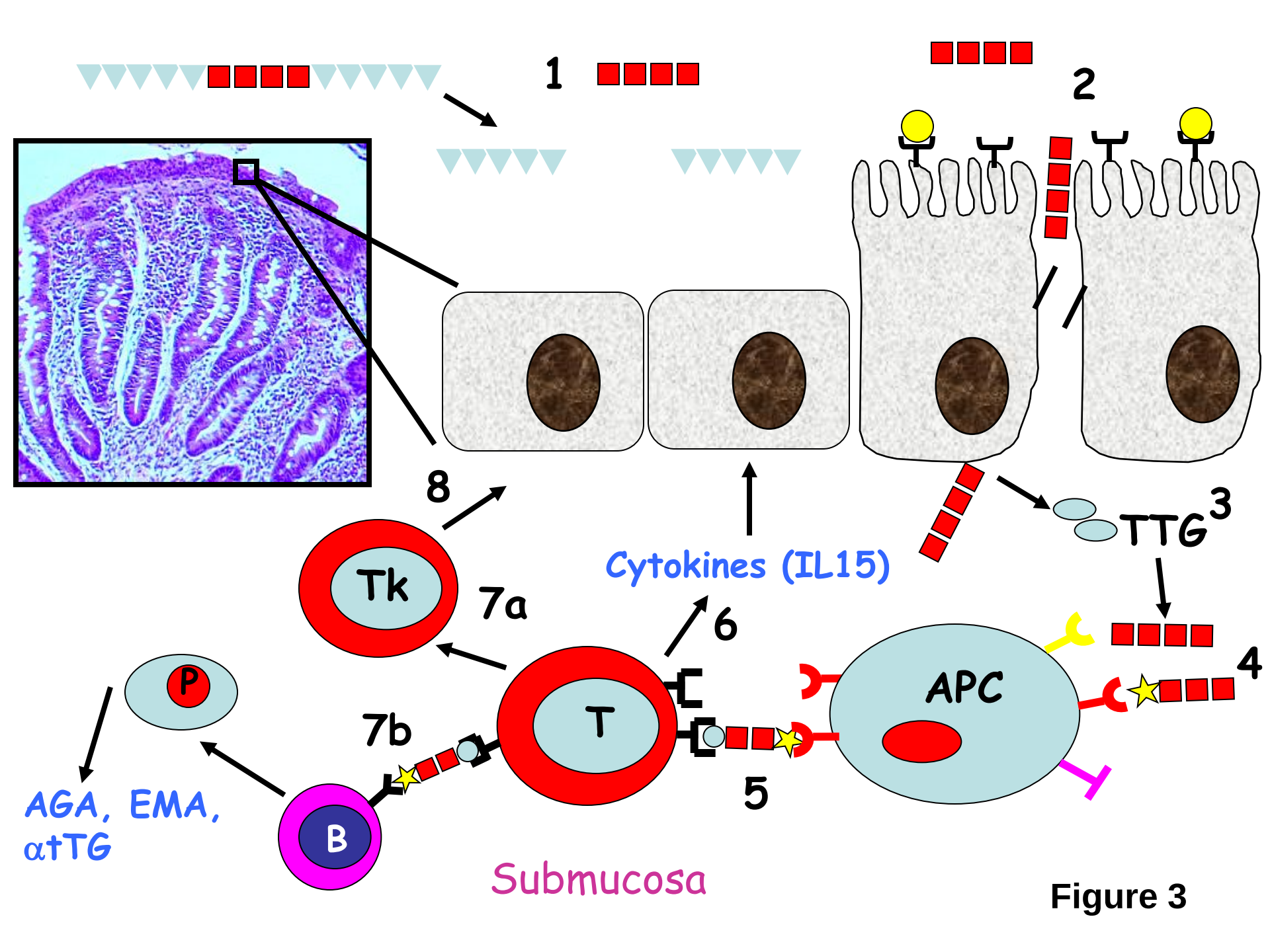

1
2
8
Tk
7a
Cytokines (IL15)
6
TTG
3
4
APC
P
7b
T
5
AGA, EMA,
αtTG
B
Submucosa

Figure 3