

Mannose-binding lectin deficiency and recurrent pregnancy loss



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Definition of unexplained recurrent pregnancy loss

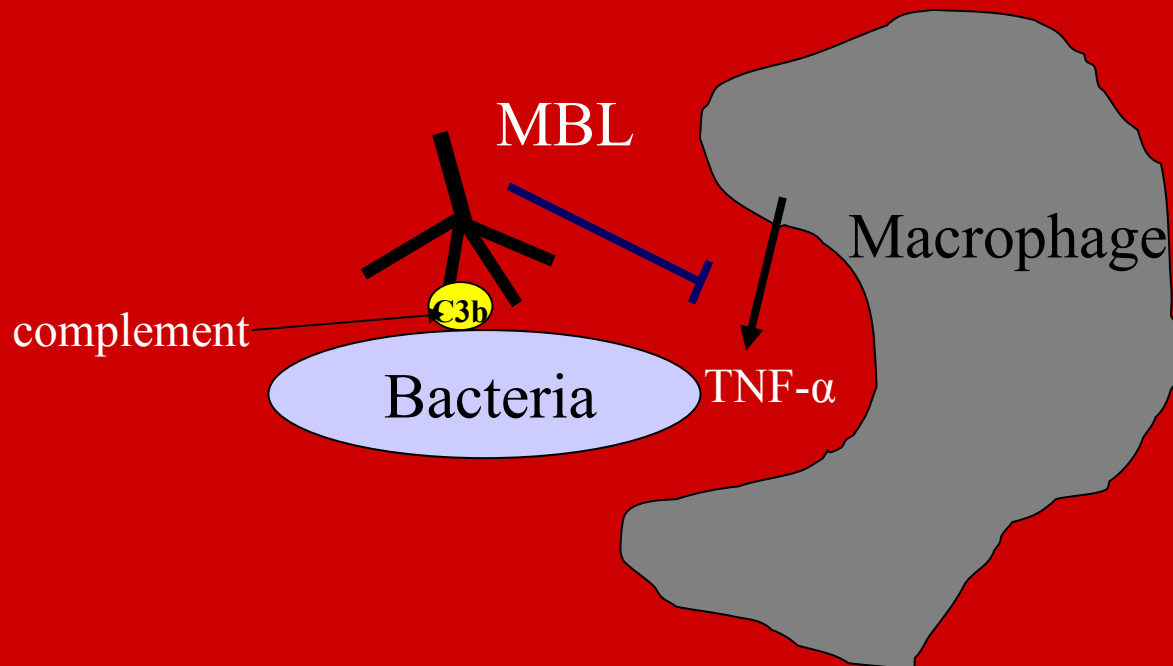
- 3 or more consecutive losses of intrauterine pregnancies before gestational week 28
- No obvious explanation of the losses after investigation of the uterine cavity, parental karyotypes, endocrine factors and lupus anticoagulant

Recurrent pregnancy loss affects
approximately 1% of all women
who attempt pregnancy

Mannose-binding lectin = MBL

MBL is a plasma lectin that acts by :

- 1) activating complement at the surface of microorganisms
- 2) promoting phagocytosis of microorganisms and apoptotic cells
(Ogden et al. JEM 2001)
- 3) modulating cytokine production (Jack et al. JID 2001)



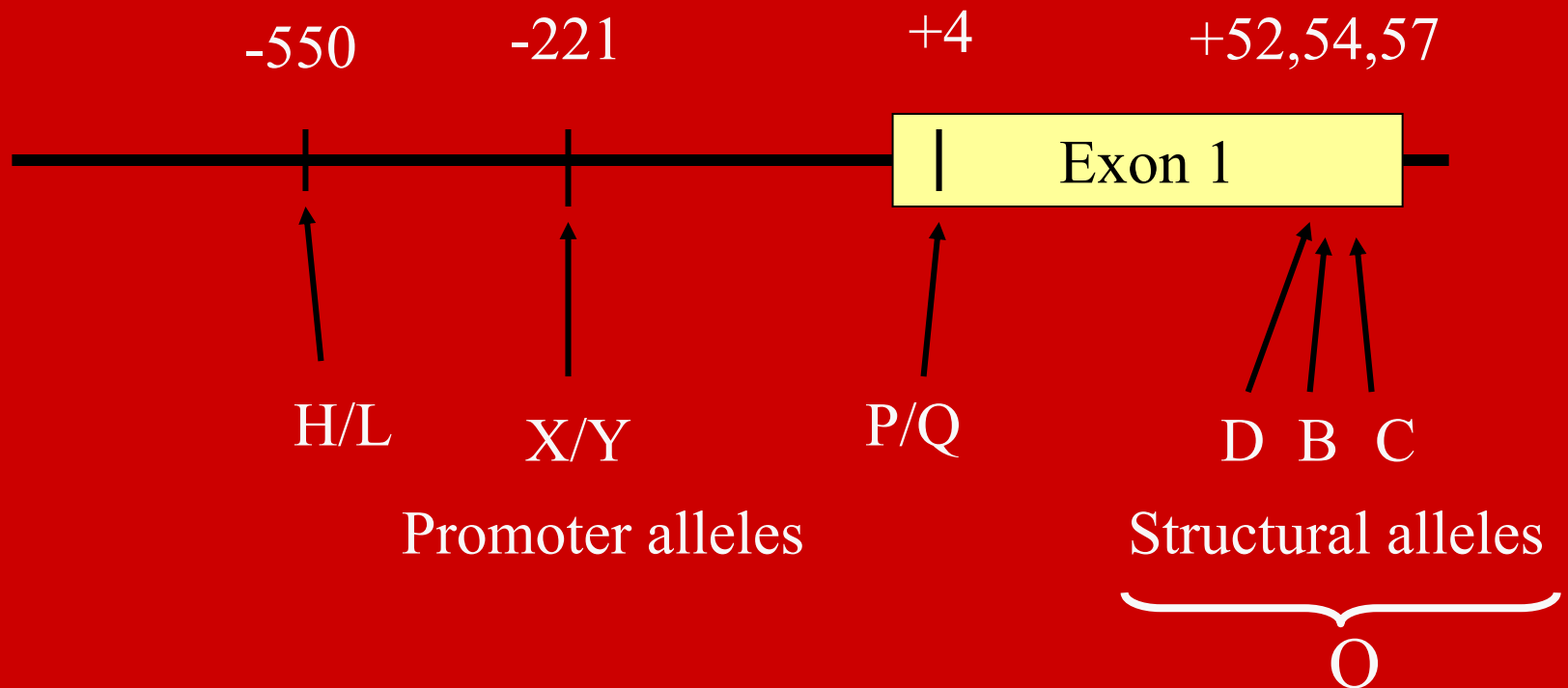
Serum MBL measurement

- **20-fold diluted serum was incubated in microtitre wells coated with murine monoclonal anti-MBL antibody. After wash, europium-labeled anti-MBL antibody was added and after wash time-resolved immunofluorescence was carried out**

Investigation of polymorphisms in the MBL gene

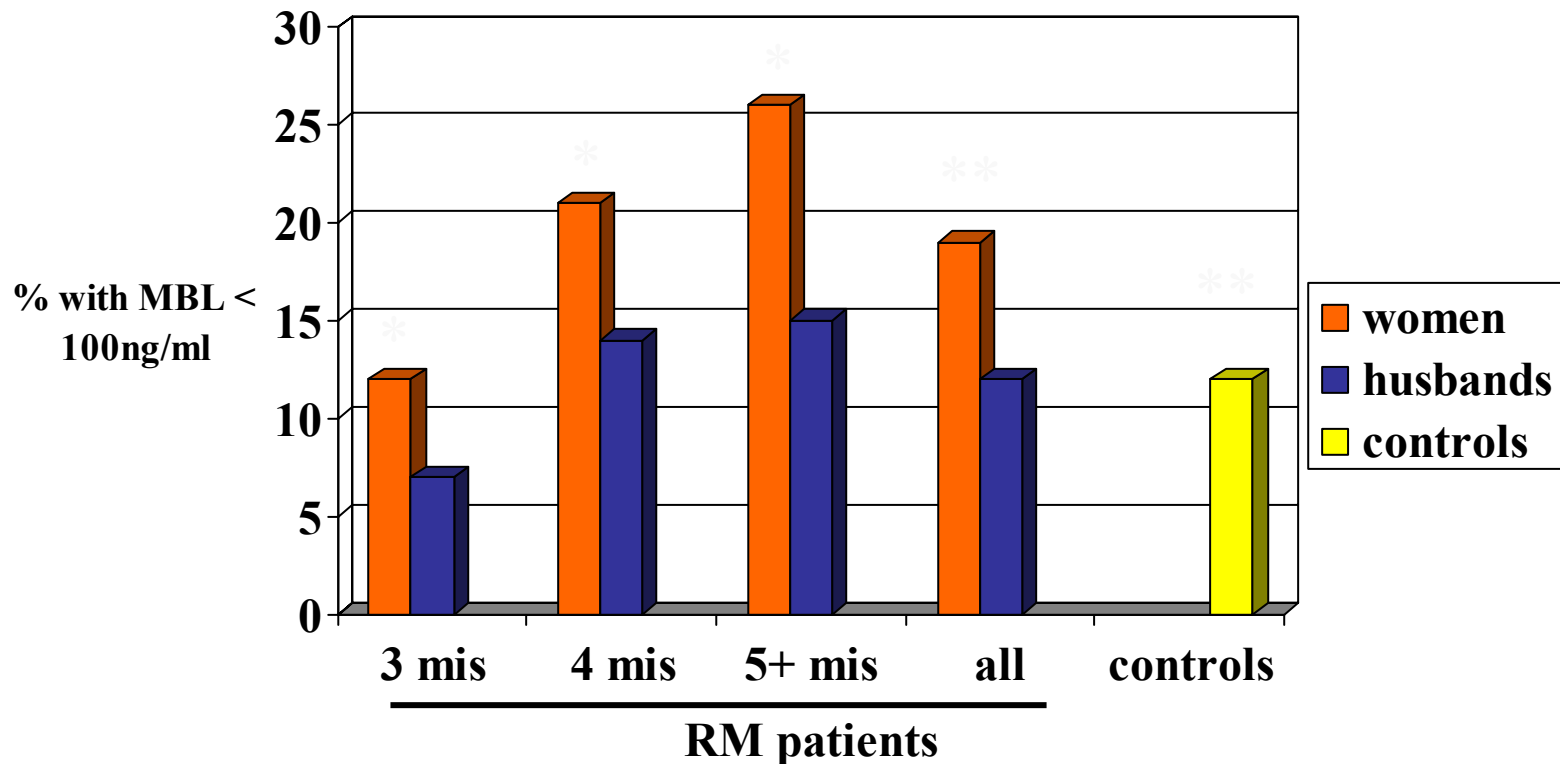
Two single nucleotide polymorphisms in the promoter region, one in the 5' untranslated region and three in exon 1 of the MBL gene were investigated by PCR-SSP technique and 12 different primers on DNA from PBL

Genetic sites on chromosome no. 10 determining MBL concentrations



Frequency of low MBL (< 100 ng/ml) in 217 RM patients, 111 of their husbands and 418 controls

(Kruse et al., Am J Obstet Gynecol 2002)



*: $p < 0.05$; **: $p = 0.02$

Frequency of MBL coding alleles A,B, C, D and promoter haplotype LX in 111 couples with recurrent pregnancy losses and 104 control couples

Allele	RPL women	RPL husbands	Fertile women	Fertile husbands
A	74%	80%	76%	79%
B	14%	13%	14%	12%
C	3%	2%	3%	1%
D	9%	5%	6%	8%
LX	19%	24%	25%	23%

No significant difference between the groups.

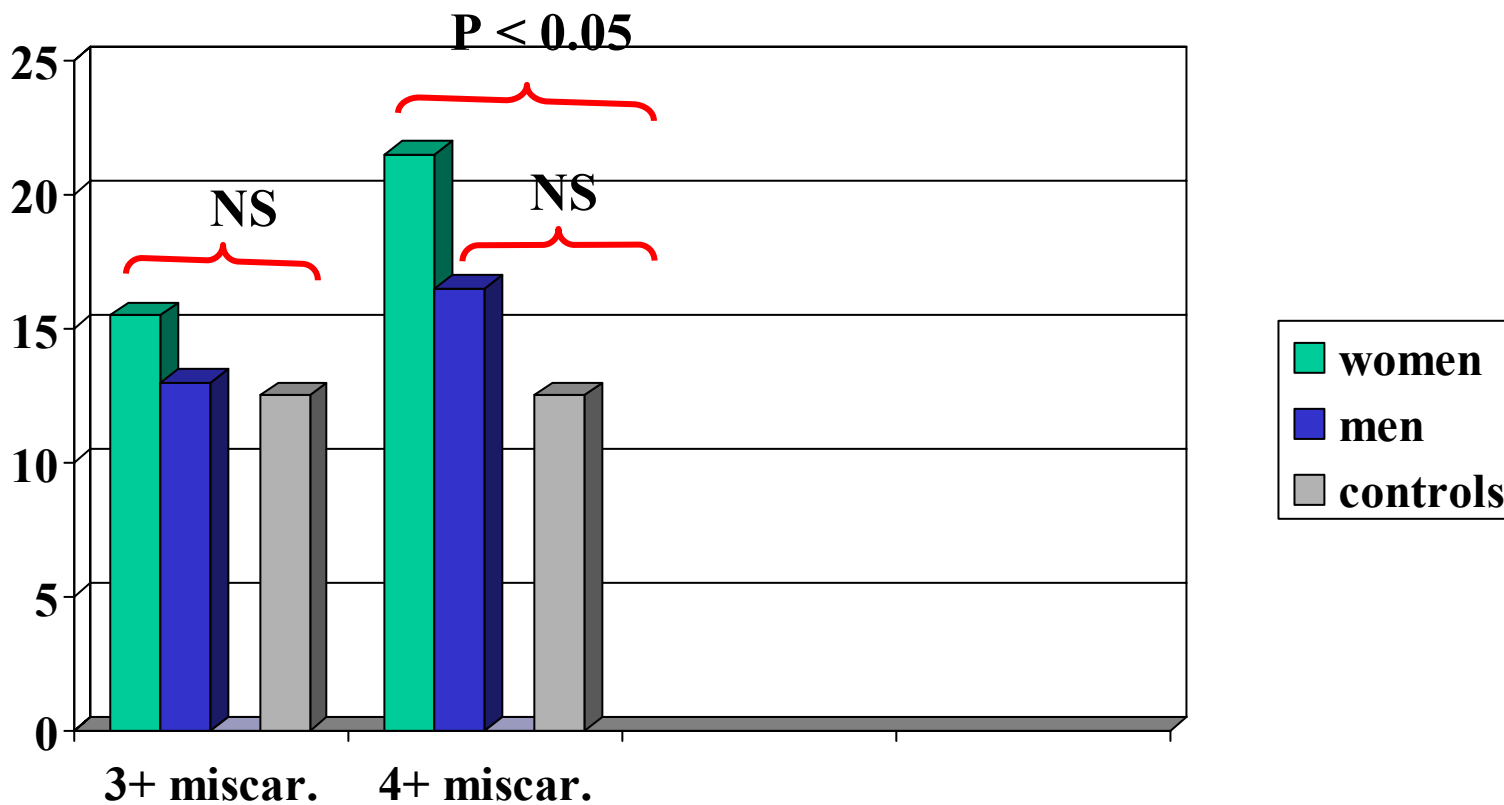
MBL genotypes associated with serum MBL levels < 100 ng/ml (Madsen et al. JI 1995)

- **Genotype O/O**

(The mutant alleles B,C and D are jointly called O alleles)

- **LXPA/O genotype**

Frequency of genotypes associated with low MBL (LXPA/O and O/O) in individuals from couples with recurrent pregnancy loss and fertile controls



An MBL level < 100 ng/ml has the same strength of association (Odds Ratio 2.1) to recurrent pregnancy loss (4 + miscarriages) as homozygosity for the MBL mutant alleles (O/O genotype) or presence of the compound O/LXPA genotype.

A negative (?) study of the association between RPL and MBL deficiency (Baxter et al CEI 2001)

Genotype	Females		Males		UK controls	P
	RPL	Controls	RPL	Controls		
	N = 76	N = 69	N = 76	N = 69		
High MBL prod	47%	56%	62%	54%	53%	NS
Medium MBL prod	37%	30%	26%	33%	32%	NS
Low MBL prod	16%	13%	12%	13%	11%	NS

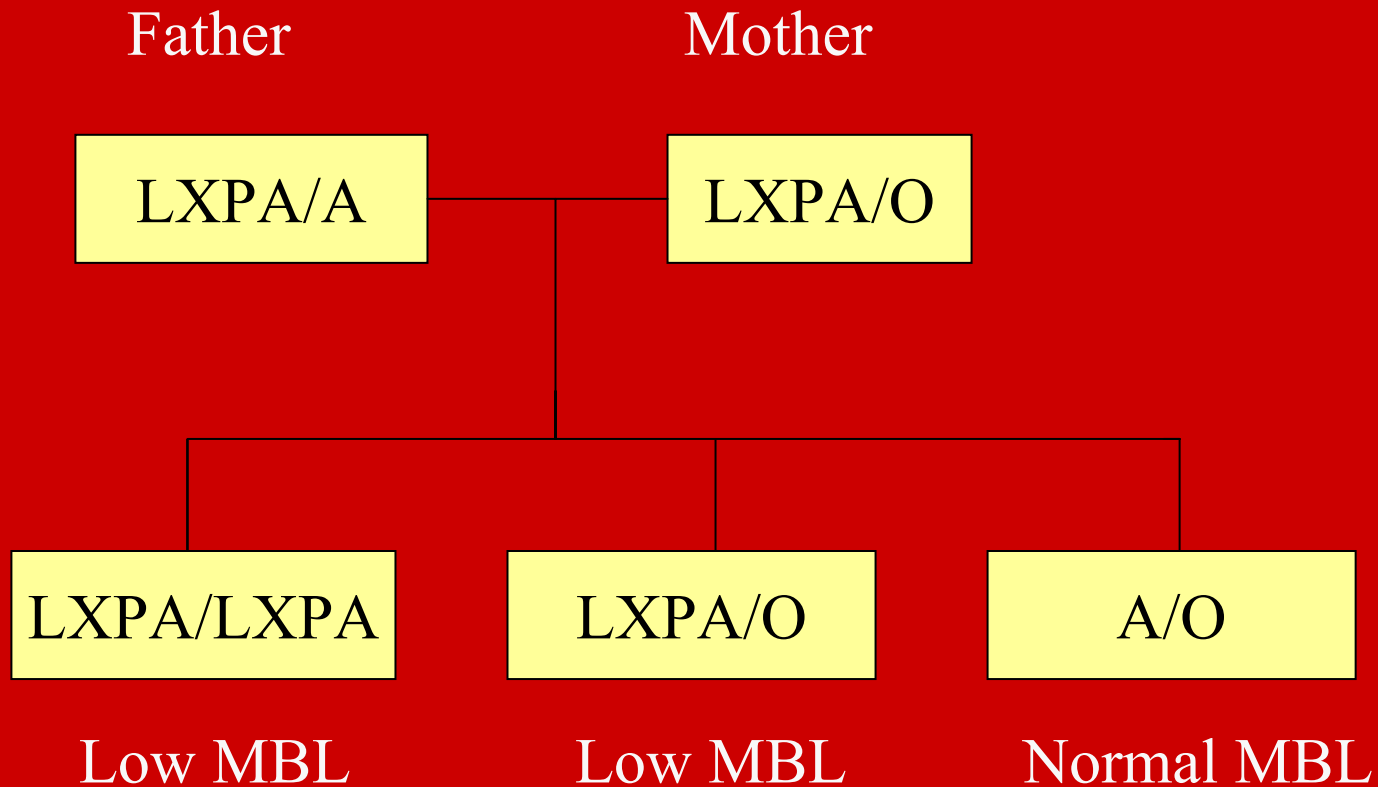
Low producing genotypes: 0/0 and 0/LXA

Kilpatrick et al (Mol Hum Reprod 1995) found that the frequency of MBL deficiency was increased in both female and male partners of RPL couples.

We (Kruse et al. Am J Obstet Gynecol 2002) found no or only weak correlation between MBL deficiency or carriage of low producing MBL polymorphisms in males and RPL among their wives

The data of Kilpatrick et al. could suggest that low fetal rather than low maternal MBL concentrations are risk factors for RPL

Inheritance of MBL genes



A = normal allele, O = mutation B, C or D

Distribution of 111 couples with recurrent pregnancy loss and 104 control couples according to their calculated risk of conceiving a fetus with a low MBL-producing genotype

Risk of fetus with genotype LXPA/O or O/O

	0%	25%	50%	75%	100%
RM couples	55%	32%	11%	1%	1%
Control couples	56%	30%	14%	0%	1%

No significant differences between the groups

Studies of serum samples and genetic analysis suggest that **only/mainly** the maternal MBL level/MBL genotype exhibit association to recurrent pregnancy loss

Outcome of the next pregnancy according to MBL levels in 172 women with recurrent pregnancy loss

miscarriages/pregnancies

	Low MBL	Normal MBL	P
Cut-off 50 ng/ml	18/28 (64%)	64/144 (44%)	< 0.05
Cut-off 100 ng/ml	21/33 (64%)	61/139 (44%)	< 0.05

Higher cut-off levels diminished the predictive value of low MBL

Summary of the evidence for the importance of low MBL in RPL

- **MBL < 50 ng/ml is associated with OR = 1.91 (p = 0.006) for RPL (Kilpatrick et al. 1999)**
- **MBL < 100 ng/ml is associated with OR = 1.68 (1.07-2.63) for RPL (Kruse et al. 2002) and a significantly decreased prognosis**
- **MBL low production alleles are associated with a population relative risk of 1.7 (0.96-3.1) of miscarriage (Dahl et al. 2004)**

Late pregnancy complications and MBL deficiency

- Impact on pregnancy outcome in the third trimester
- Impact on pregnancy outcome in the second trimester

Perinatal data of the first pregnancy that progressed to > 28 GW in RPL patients according to MBL levels

	MBL < 100 ng/ml	MBL > 100 ng/ml	P
	N = 21	N = 121	
Median birth weight	3050g	3250g	0.06
Median birth weight > 37th GW	3100g	3397g	0.03
Preterm birth < 37th GW	14%	14%	NS
Perinatal mortality	5%	7%	NS

RM patients with repeated losses after cervical dilatation and rupture of membranes

Name	Miscarriages (GW)	MBL	Other	Outc
JGS	23,16,8,21,17,8	1464		birth
PMO	Livebirth, 20,21,24	2	Amenorhea	
HT	17,17,9,15	98		
AB	6,14,16,16	7786	Cerclage x 1	
GEK	17,18,18,10	263	Cerclage x 1	
KPM	22,22	6		birth
SSE	12,18,9, Liveb.36, 5,21,15,8,23	17	IDDM	birth
CH	8,7,12,21,18,17	8	Ut. sept. resection	
GKR	18,23,16,11	709	Re. lung infect., cercl. x 1	
SCS	Livebirth, 23, 21,14,21	1140	IVF, cerclage x 1	

5/10 = 50% of patients vs. 51/418 = 12.2% of controls; $p < 0.0005$

Study of interaction of HLA-DR3 and the low MBL phenotype

- In 355 Caucasian women with at least 3 consecutive unexplained miscarriages both HLA-DR determination and MBL measurement were undertaken

Background and aim of the study

- Our group has provided strong evidence that the HLA-DR3 allele and the mannan-binding lectin deficiency phenotype are associated with recurrent miscarriage
- Is it possible to demonstrate an epistatic effect between these two genetic factors with regard to susceptibility to recurrent miscarriage?

Definition

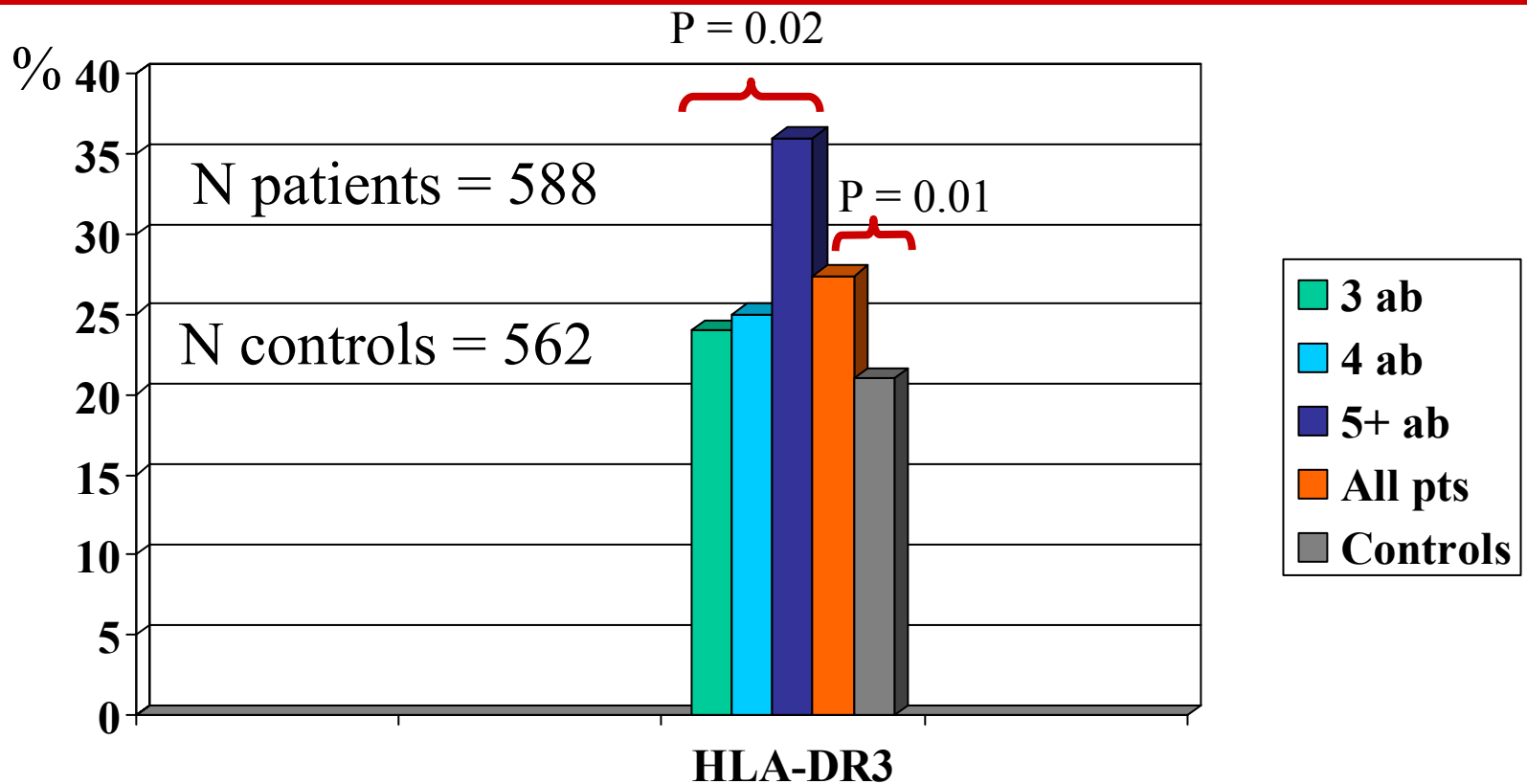
- Epistasis: the interaction between two or more genes to control a single phenotype

Examples of epistasis

Coagulation factor alleles FII 20210A and FXIII-A Leu34 alleles display a synergistic effect that strongly predisposes to myocardial infarction (Butt et al. 2003)

The combined HLA-DR3, TNF-alpha-308A, IL1alpha -889C/C genotype interacts to yield a strong association (OR = 8.0) for systemic lupus erythematosus in Caucasians (Parks et al. 2004)

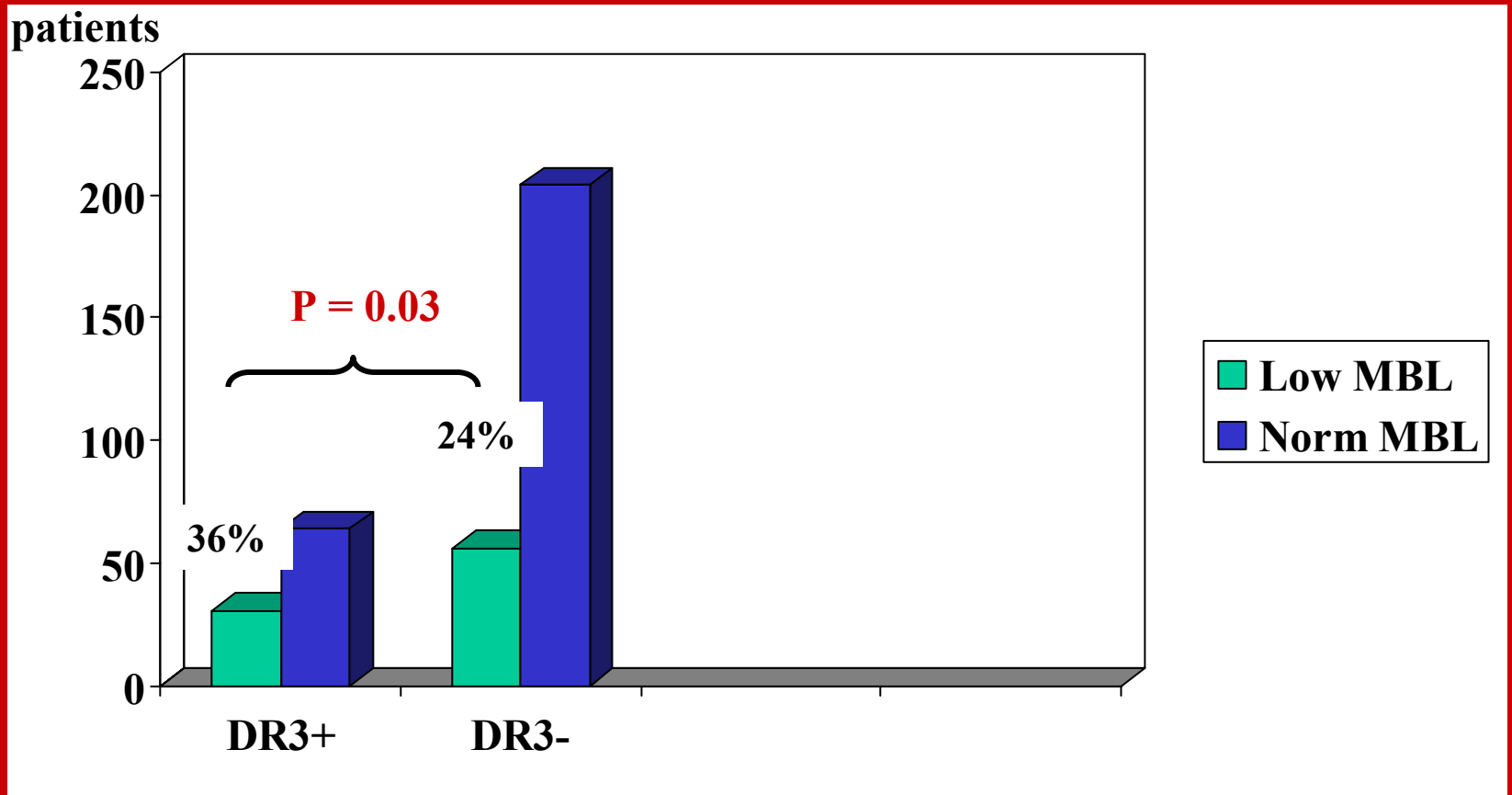
Association between maternal HLA-DR3 positivity and recurrent miscarriage (Kruse and Chistiansen, Hum Reprod. 2004)



Possible background for the associations between HLA-DR3 and recurrent pregnancy loss

- Linkage disequilibrium between high-producing TNF-alpha polymorphisms and HLA-DR3
- Linkage disequilibrium between HA-DR3 and HLA-G alleles associated with low soluble HLA-G production

MBL levels in 355 Caucasian recurrent miscarriage patients according to being positive or negative for HLA DR3



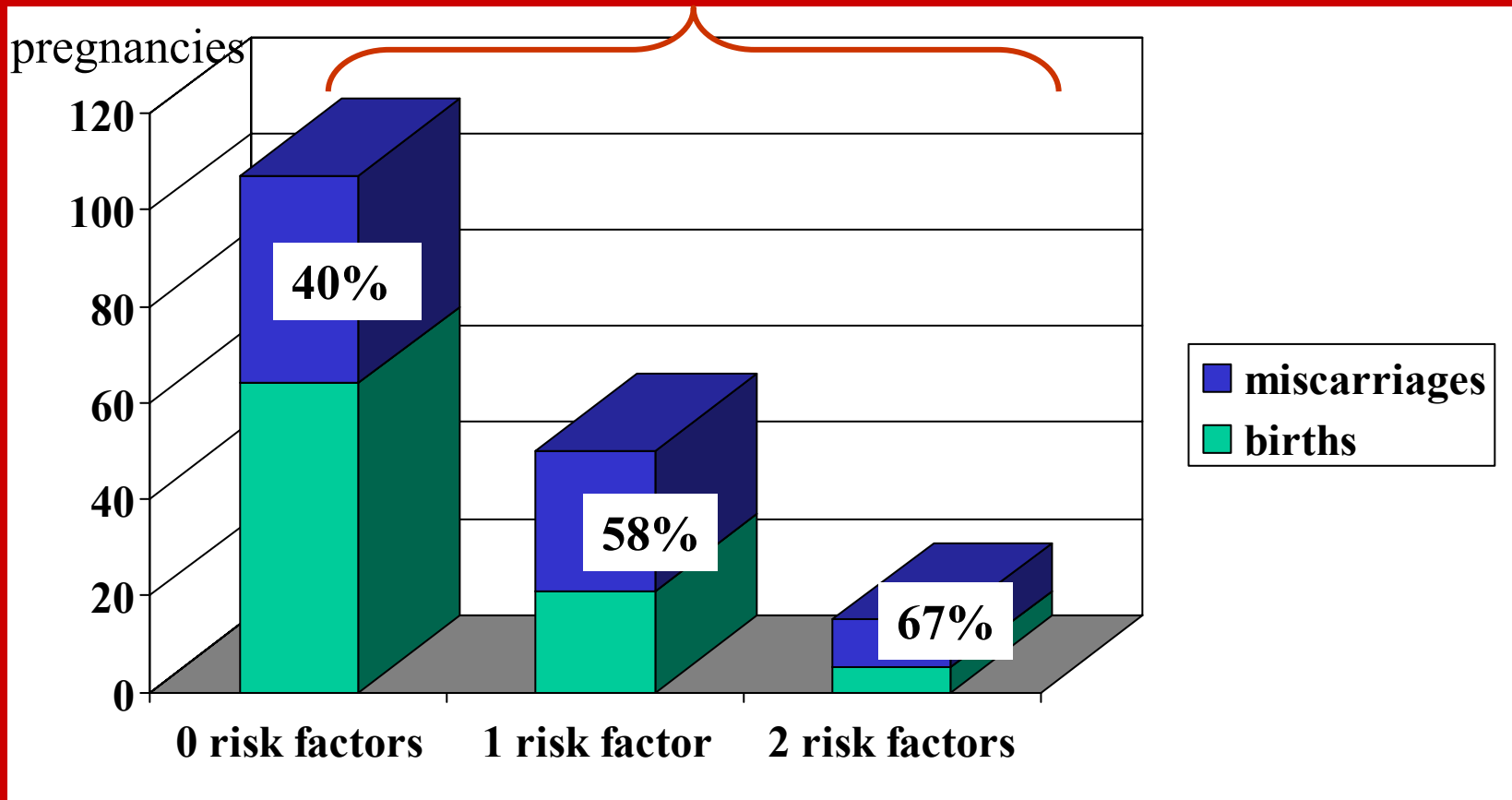
Outcome of 1st pregnancy after referral according to HLA-DR3 and serum MBL level in 172 women with recurrent miscarriage

births/pregnancies	HLA-DR3 neg	HLA-DR3 pos
MBL \geq 100 ng/ml	64/107* (60%)	14/32 (44%)
MBL < 100 ng/ml	7/18 (39%)	5/15* (33%)

* P = 0.05

Miscarriage rate in the first pregnancy after referral in 172 patients with recurrent pregnancy loss according to the presence/absence of HLA-DR3 and MBL < 100 ng/ml

P = 0.01



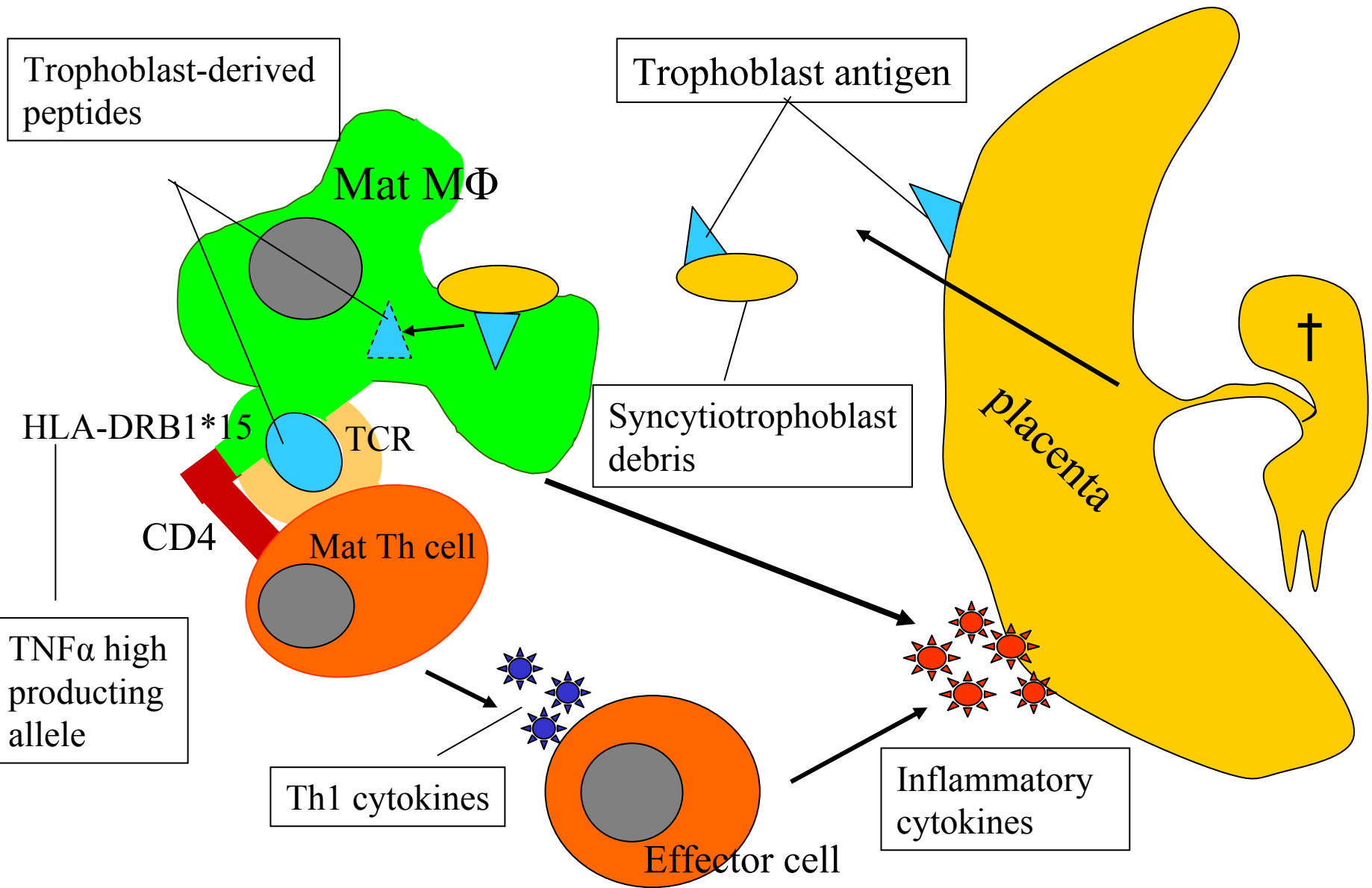
Conclusions

- The tendency for a higher risk for recurrent pregnancy loss in women carrying both HLA-DR3 and genes for MBL deficiency and the significantly increased risk of subsequent miscarriage in women carrying both risk factors may indicate epistasis between the HLA region and mannose-binding lectin

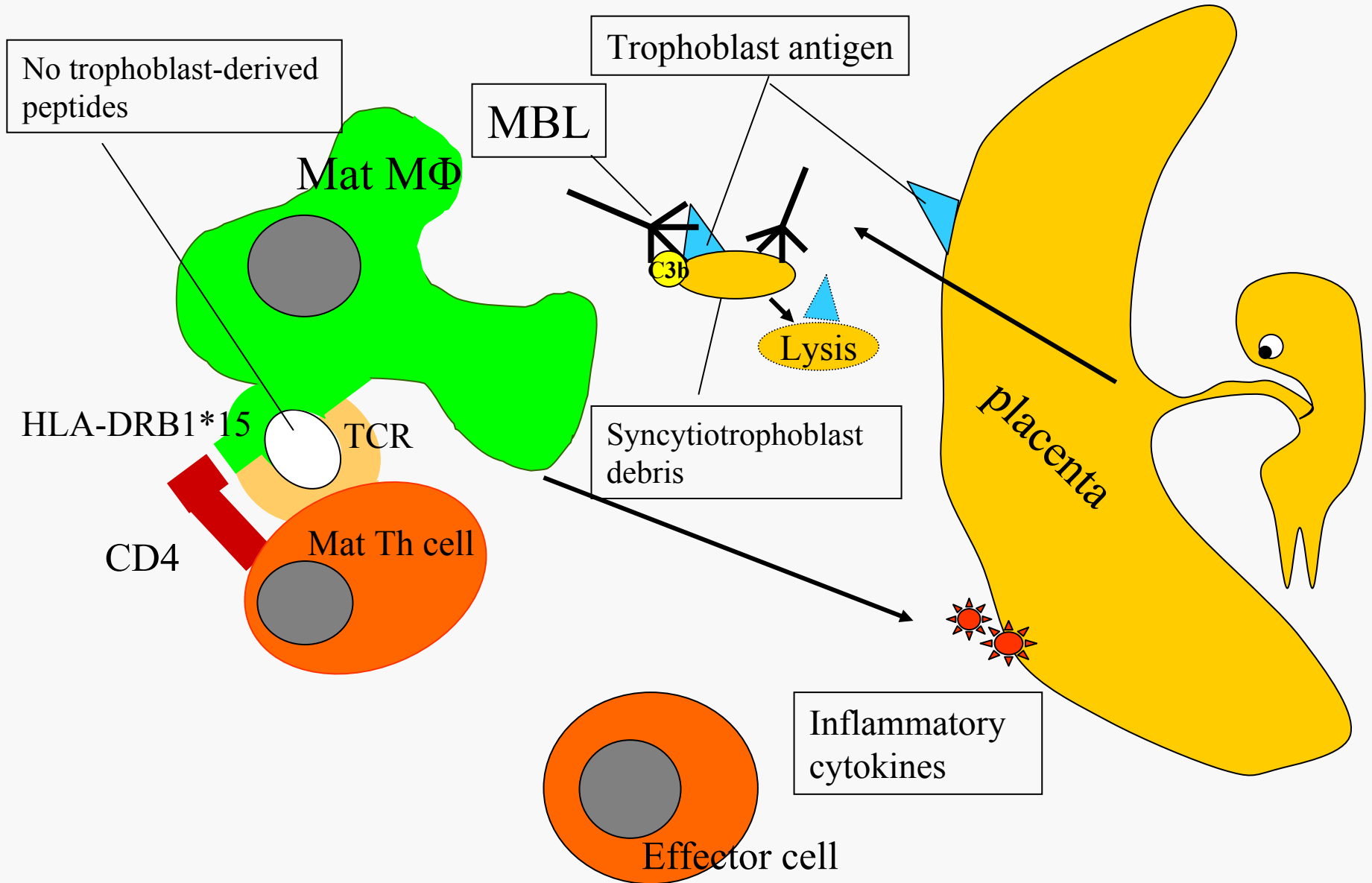
Assumptions for hypothetical pathophysiologic scenarios at the feto-maternal interface explaining the epistasis between HLA-DR3 and MBL deficiency in RPL

- **Some inflammation at the feto-maternal interface is beneficial for placental invasion and normal pregnancy**
- **Excessive inflammation results in miscarriage, preterm birth or fetal growth restriction**
- **Apoptotic syncytiotrophoblast debris shed from the placenta induces various degrees of inflammation dependent on the amounts shed**

Woman with MBL deficiency



Woman with normal MBL levels



Conclusions

- Several studies have documented that maternal MBL levels $< 100\text{ng/ml}$ or maternal carriage of low producing MBL genotypes are risk factors for recurrent pregnancy loss
- The association to recurrent pregnancy loss is most pronounced in patients with 4 or more previous miscarriages
- Paternal or fetal MBL levels/genotypes seem not to play a role for recurrent pregnancy loss

Conclusions

- Low maternal MBL levels also seem to be a risk factor for a new miscarriage, second trimester pregnancy loss and low birth weight in successful pregnancies

Conclusions

- The pathogenesis of recurrent pregnancy loss is probably multifactorial
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- Low MBL levels must be considered one of a series of factors being responsible for an increased level of inflammation at the fetomaternal interface and thereby increasing the risk of recurrent pregnancy loss

Thank you for your attention