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Prospects for the use of hepatitis C virus envelope protein highly conserved sites in peptide vaccines.

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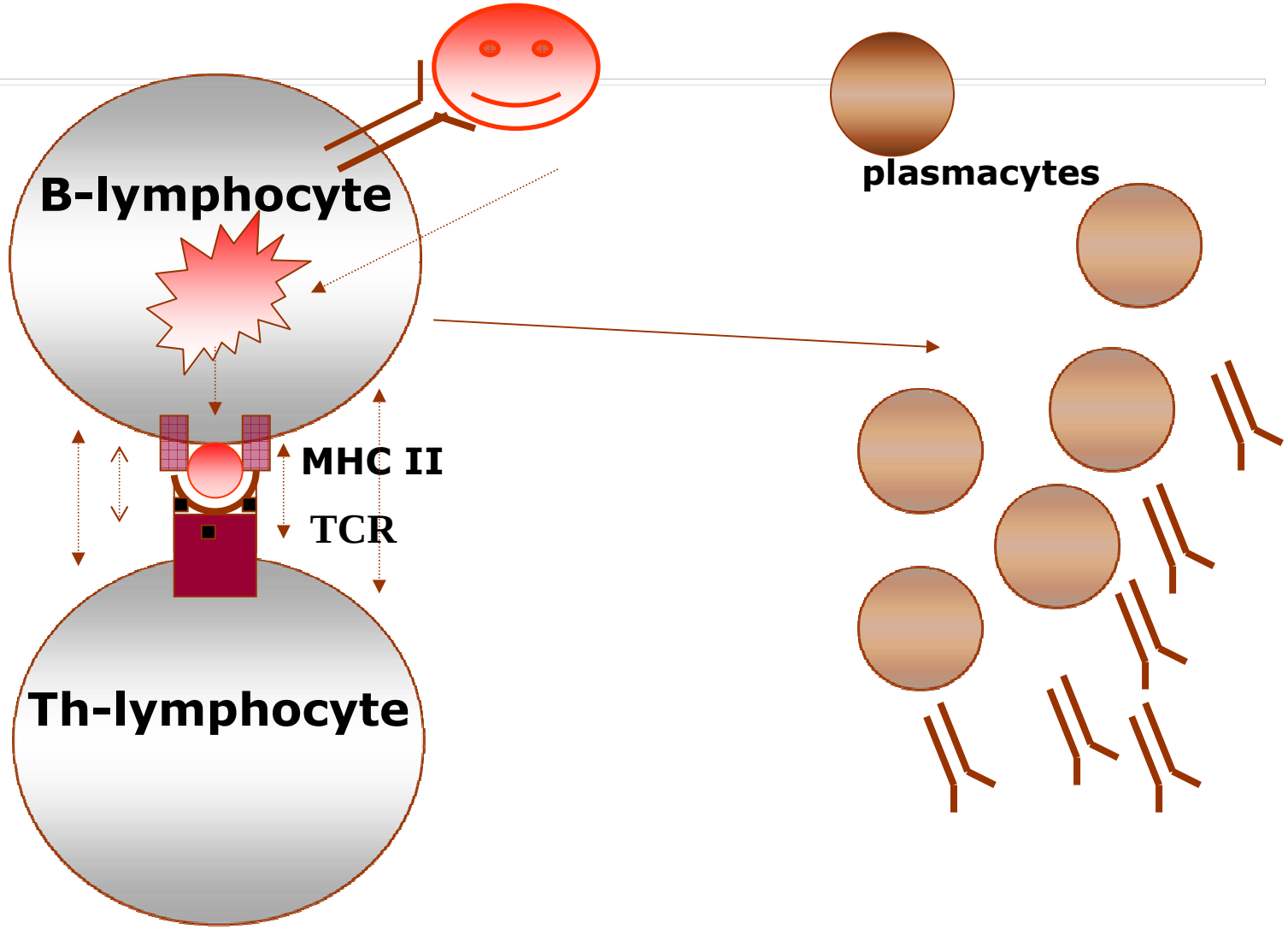
Specific features of HCV posing problems for the traditional vaccine development

- Absence of large-scale biotechnological methods for virus production
- Extremely high genetic variability and inhomogeneity of HCV isolates
- Insufficient data on functionally important sites and antigenic determinants of HCV proteins
- HCV binding to and persistence in immune system cells that influences host immune responses
- Absence of standard experimental (cell culture, small animal) models of HCV infection
- Retarded and inefficient immune responses to HCV proteins
- Mechanisms of HCV entry into cells almost unknown

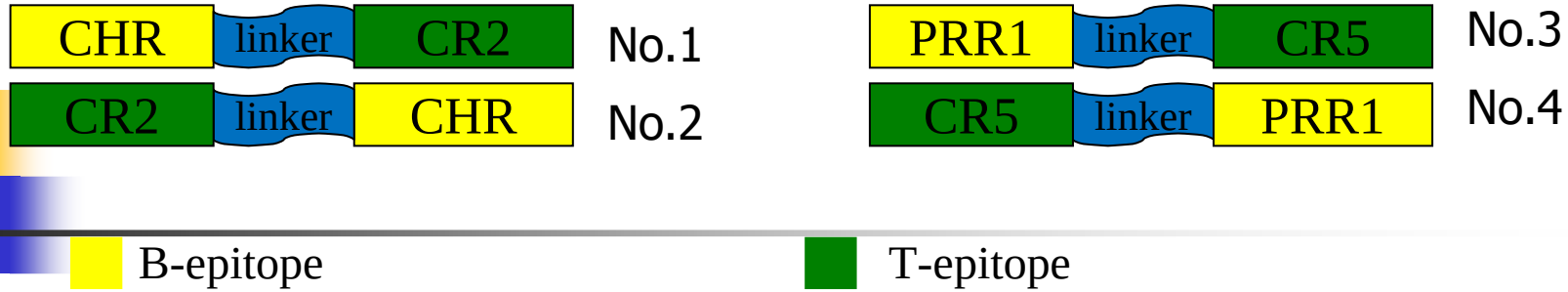
Aim of the study:

Design of synthetic peptide immunogen constructions based on fragments of HCV envelope protein E2, which should induce antibodies capable to interact with full-size HCV envelope proteins and virions

Th-B interaction



T-B epitope construct



B-epitope T-epitope

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          C >< E1
LlSCltiPasayevrNvsglYhvTNDCsNsSIvyeaadmilHtPGCvPCvreg-NsS-RcWvaltPtlAarnasvpttti

                                                                CR1
RrHvDllVgaAafCSAmYvGDlCGsvfLvsQlFtfsprrhetvQdCNCSiYpGhvtGHRMAWDMMmNWsPttalvvsqll

                                          E1  >< E2
RiPqavvdmvaGaHWGvlaglaYysMvgnWAKVlivlllfaGVda----eThvtGgsaarttsgltslfspGakQniqLi

        GLR                                                                PRR1
NtNGSWHINrTALNCNdSlqTGflAaLfYthkFNssGCpeRmasCrpidkFaqGwGpityaepng----sdqrPYCWHYa

                    CR2                                         PRR2
 PrpCgivpAsqVCGPVYCFTPSPVVVGTTDrsGaPTYsWGeNetDVllLnntRPPqgnWFGCTWMNsTGftKtCGgPPCn

                     CR3                            CR4
Iggvg-----Nnt---LtCPTDCFRKHPeATYtkcGsGPWlTPrClVdYPYRLWHYPCTvNftifKvRMyV-GGvEHRln

              CHR                              CR5
AACNwTRGeRCdleDRDRselsPLLlsTTewqiLPCsfttlPALSTGLiHLHqNIVDvQYLYGvgsavvsfaikWEyvlLlFllL

               E2 >< p7
ADARvCacLWmmlliaQaEAALENLvvLNAasvAG
```

Consensus sequence of HCV envelope proteins derived from 827 individual aligned sequences

Titers of antipeptide antibody in serum samples of laboratory animals immunized with T-B epitope constructs

Mice Immunization Injection 20 mkg per animal, 4 injections with 2-week intervals

№ group	Immunogen preparation	Geometric mean antibody titer values in animal sera
1a	Peptide 1 without FA	10 090
1	Peptide 1+ FA	256 000
2a	Peptide 2 without FA	149
2	Peptide 2 + FA	21 000
1-BSA	Peptide 1-BSA + FA	48503
2-BSA	Peptide 2-BSA + FA	2639

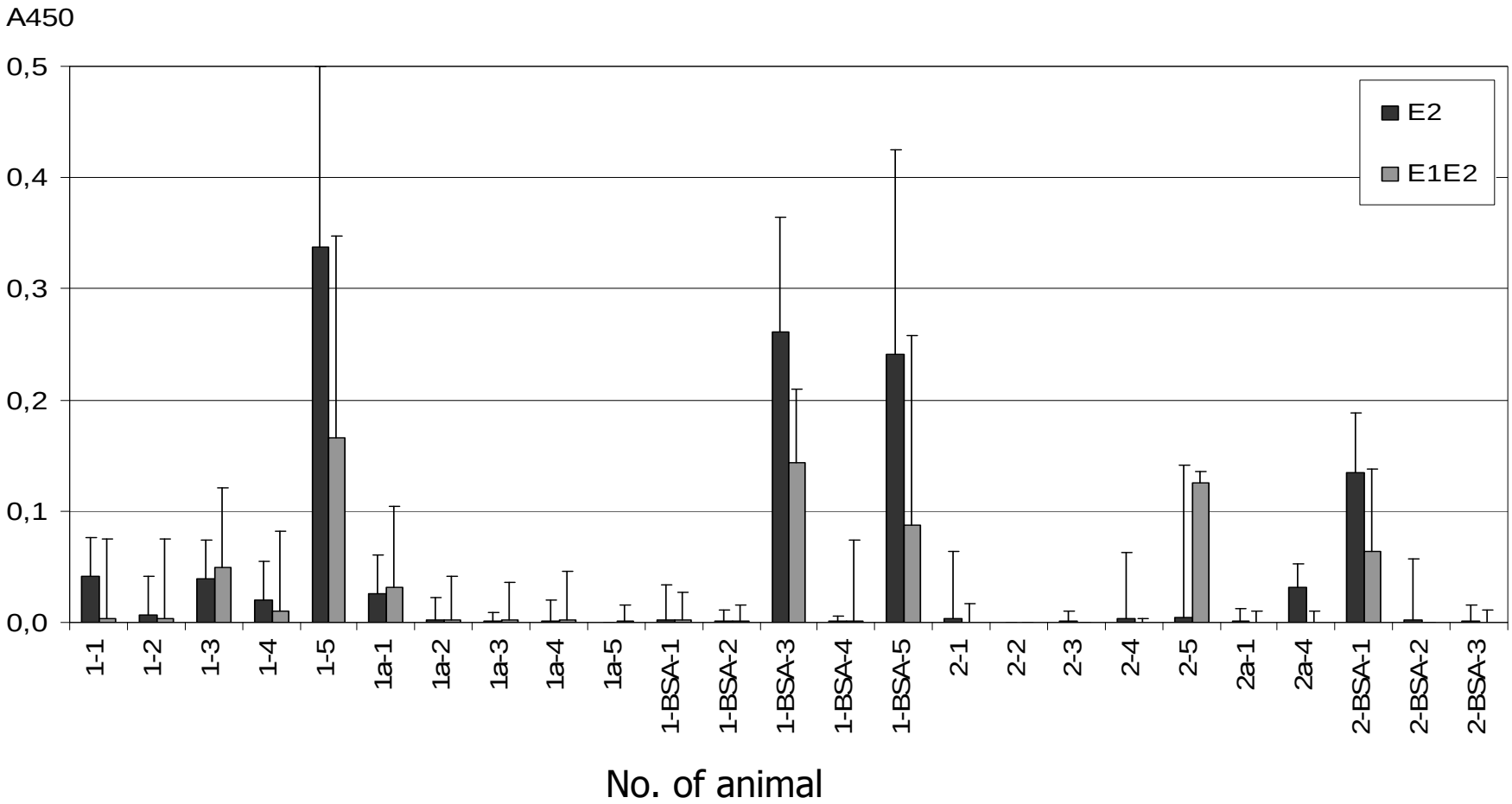
Rat Immunization Injection 100 mkg per animal, 4 injections with 2-week intervals

№ group	Immunogen preparation	Geometric mean antibody titer values in animal sera
1	Peptide 1+ FA	5616
2	Peptide 1 without FA	<100
3	Peptide 3 + FA	<100
4	Peptide 3 without FA	<100
5	Peptide 4 + FA	<100
6	Peptide 4 without FA	<100

FA-Freund's adjuvant

BSA- bovine serum albumin

Interaction of mice serum antibodies against T-B-epitope constructs with HCV envelope protein E2 and E1E2 heterodimer



Immunogenicity of construct 1 in immunization conditions close to real vaccination

Mice
Immunization
Injection
176 ng per animal,
3 injections with 4-week intervals

№ animal	Geometric mean of antibody titers values in serum in this group					
	14 days*)		58 days*)		119 days*)	
1	300000	122474,5	25000	25000	5000	5000
2	100000		25000		5000	
3	25000		25000		5000	
4	300000		25000		5000	
5	25000		25000		5000	

*) – period after the last injection

Conclusions

- Highly conversed fragments of HCV E2 envelope protein designed as artificial peptide immunogen constructs show high immunogenicity and induce specific antibodies that interact with full-size HCV envelope proteins E2 and E1E2. taking into account the conserved structure of the fragments, the viral isolate non-specificity of the induced antibodies is supposed.
- Such peptide immunogenic constructs can be the basis of the anti-HCV synthetic candidate vaccine.

Acknowledgments

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