Mutants and HBV vaccination

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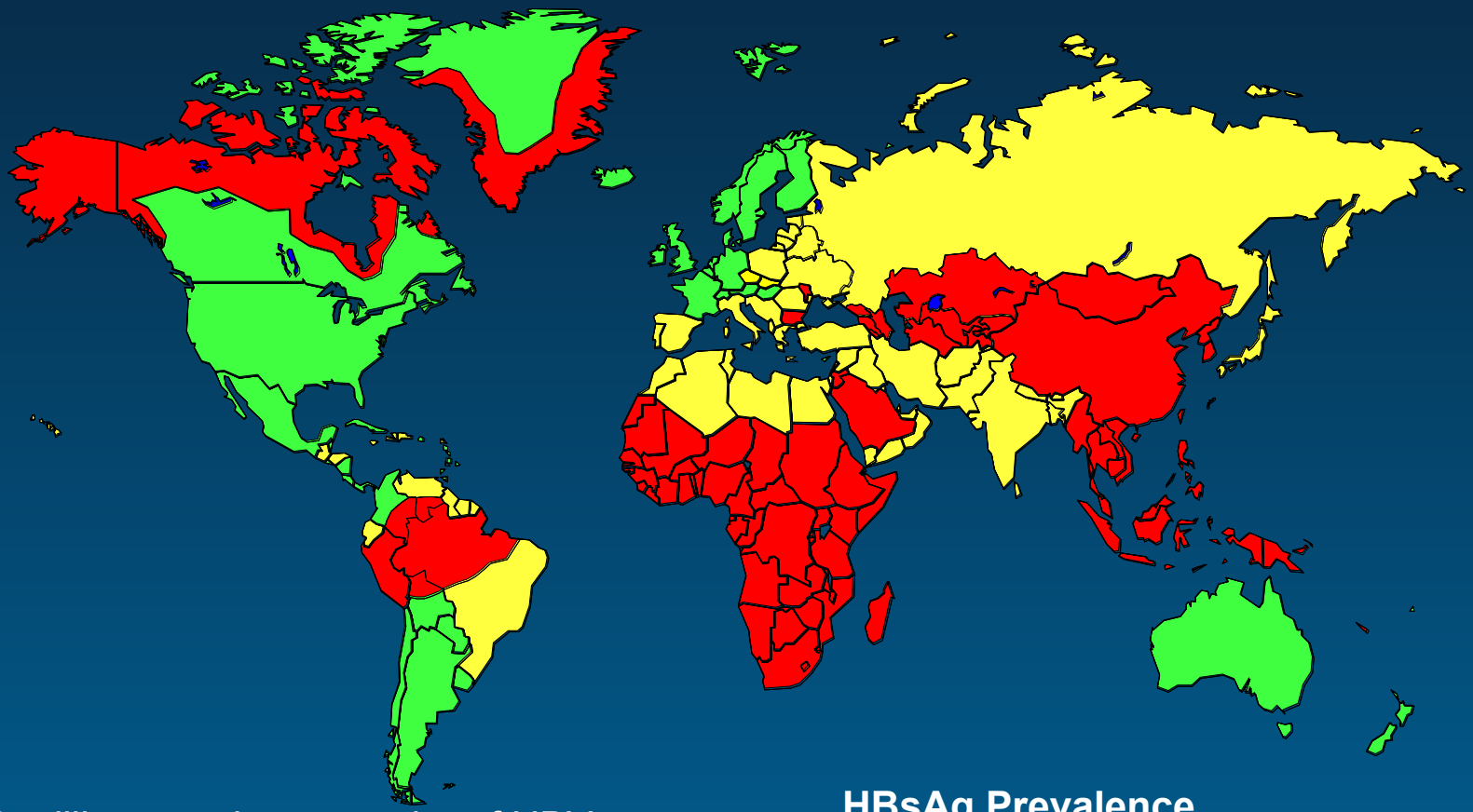
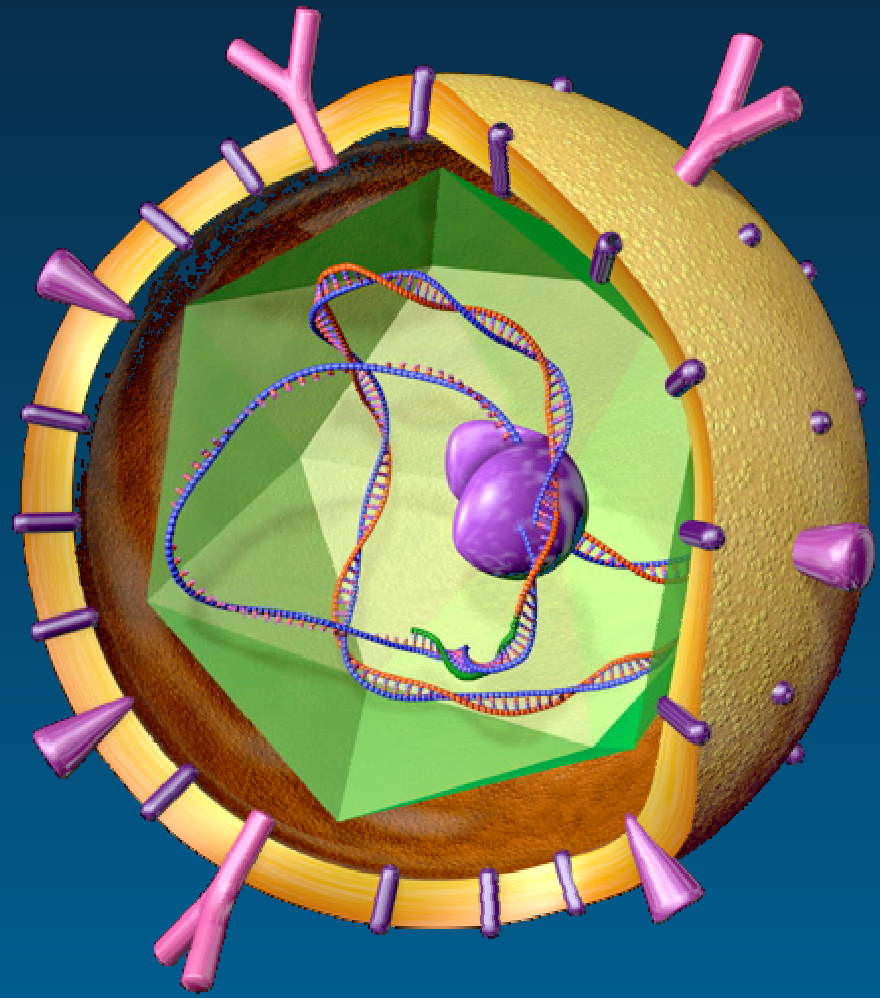
Geographic Distribution of Chronic HBV Infection



- 400 million people are carrier of HBV
- Leading cause of cirrhosis and HCC
- 9th major cause of death
- 40% of death are related to HBV in vertically infected people

HBV and mutation

- Although HBV is a DNA virus, replication is through an RNA-replicative intermediate.
- The estimated mutation rate is approximately 1nucleotide/10,000 bases/infection year. (10 fold higher than other DNA viruses)
- Therefore 10^{10} mutations enter the virus pool daily.
- Overlapping reading frames restrict the mutation rate.



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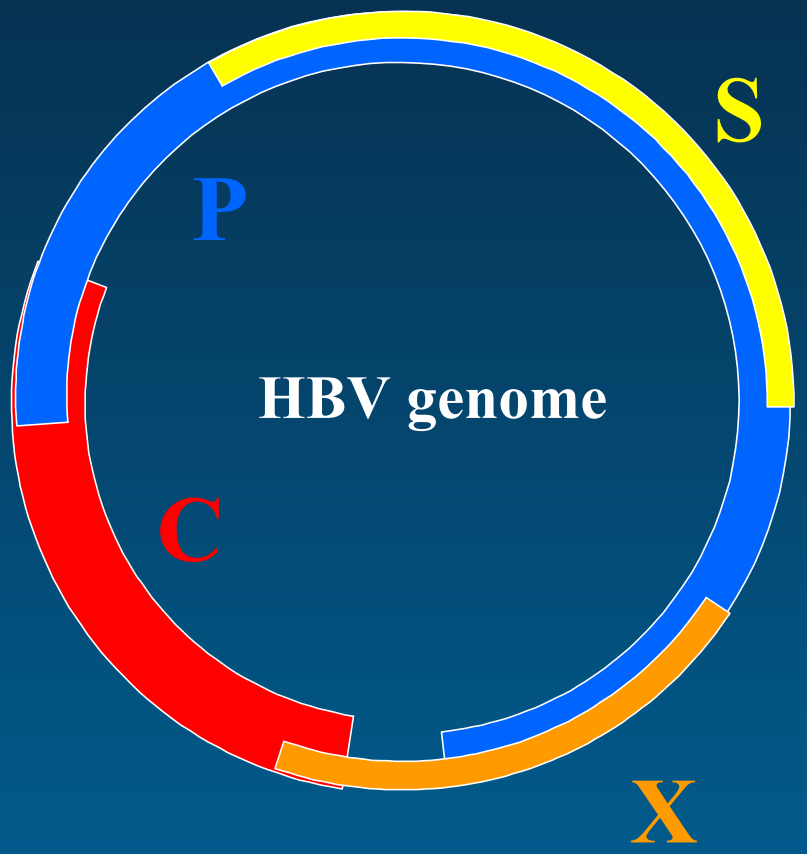
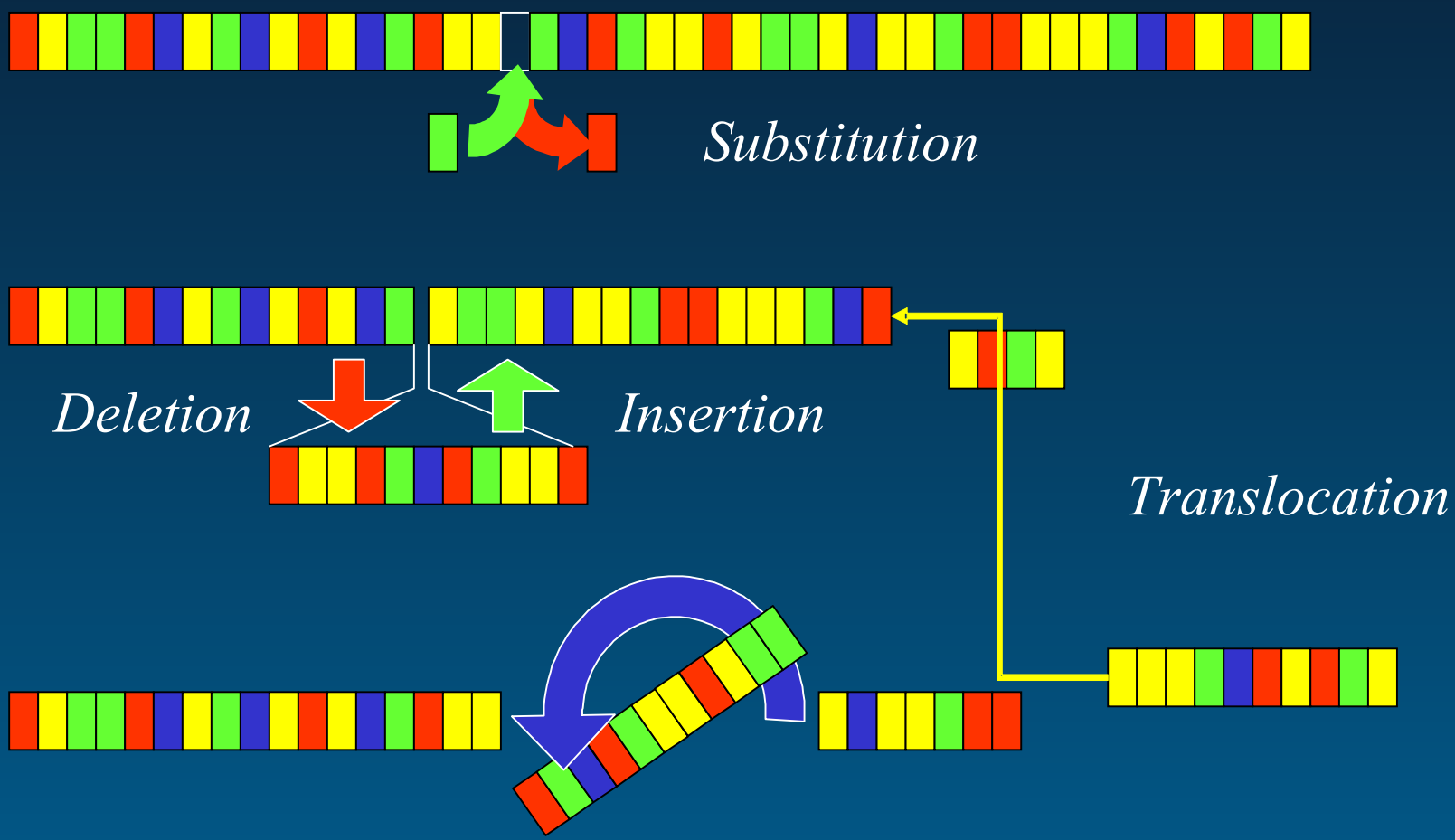



Substitution
Deletion
Insertion
Translocation
Inversion

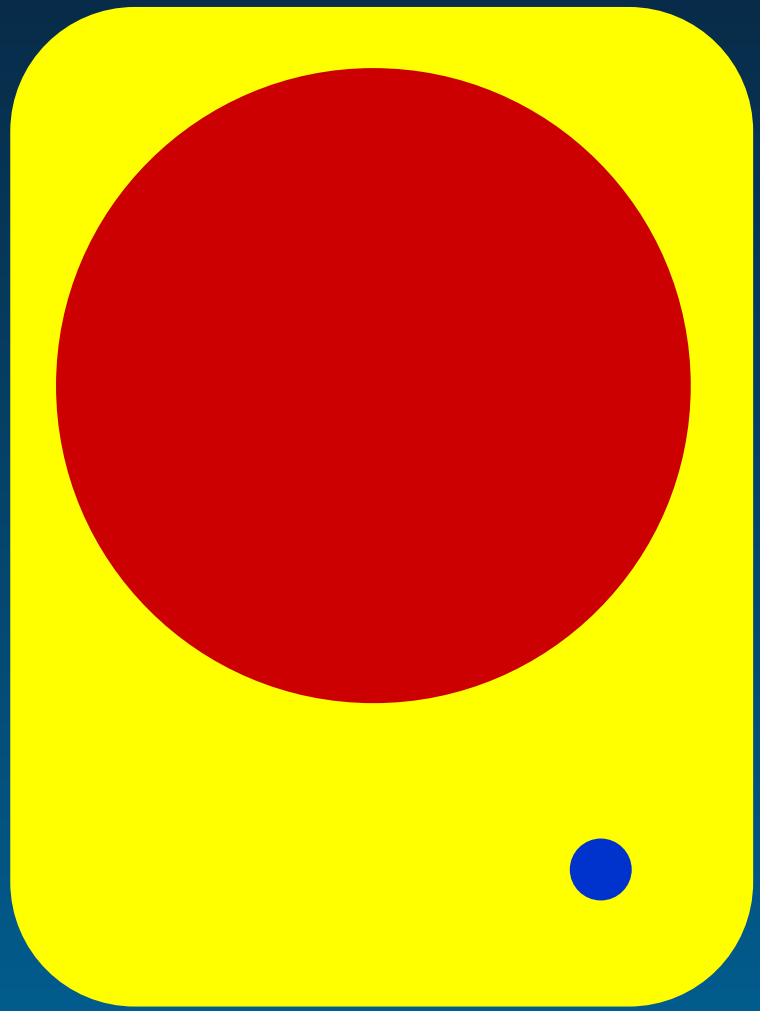
HBV and Mutation

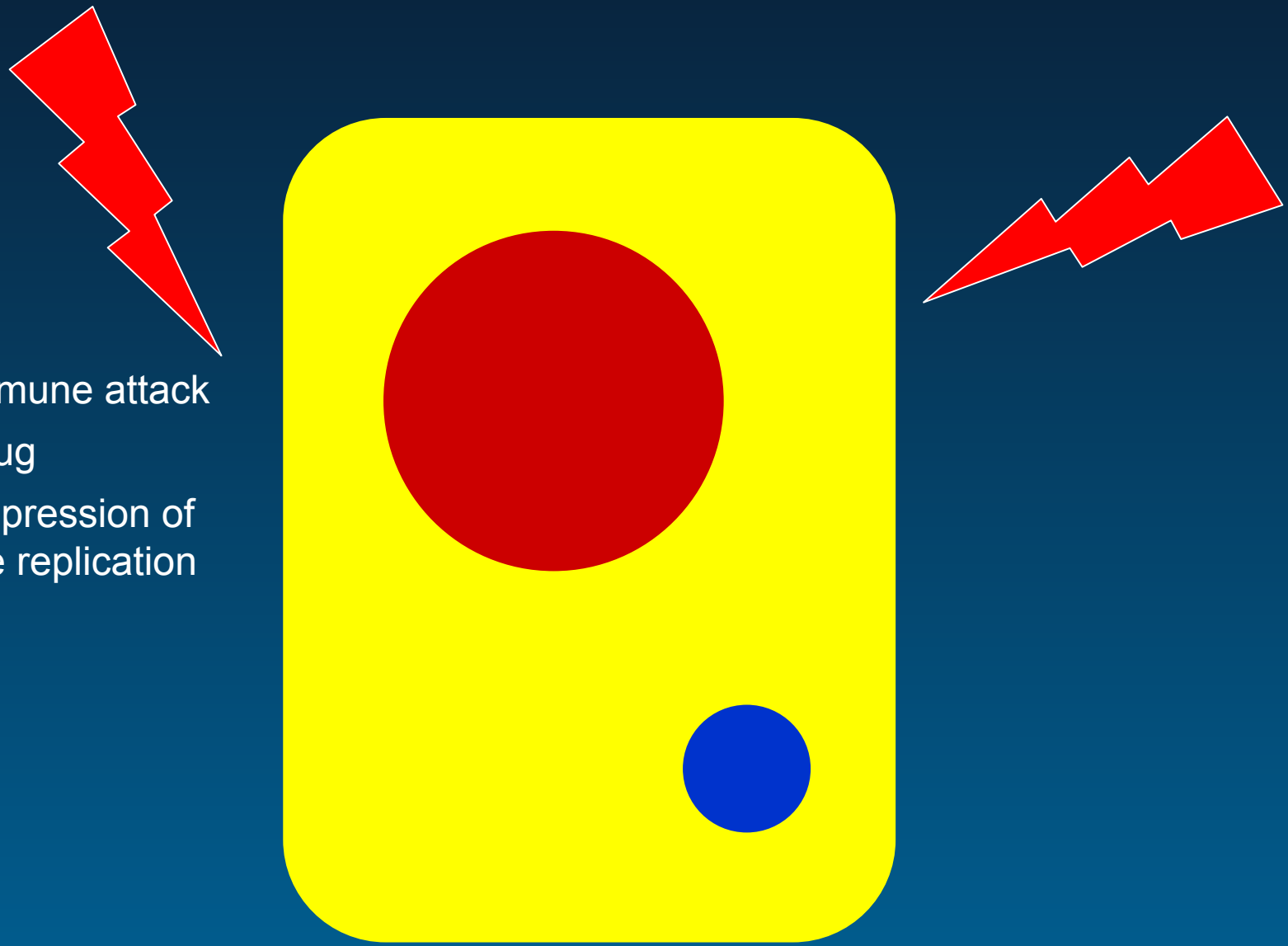
- Naturally occurring mutations should provide some advantages to the organism.
- The possible advantages for HBV:
  - Increase in replication effectiveness
  - Escape from immune recognition
  - Resistance to antivirals
  - Facilitation of the steps in HBV life cycle

Immune attack

Drug

Repression of the replication

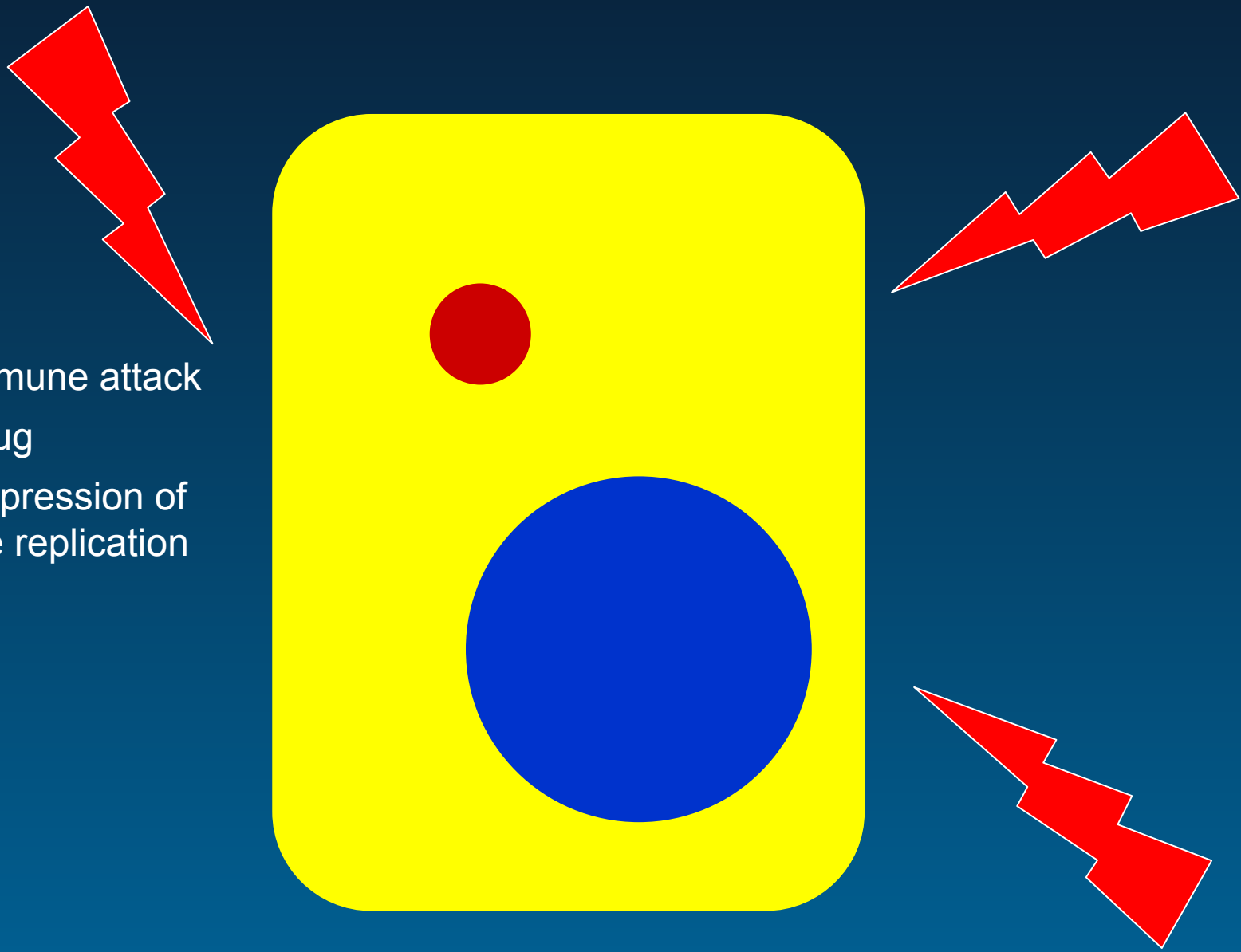


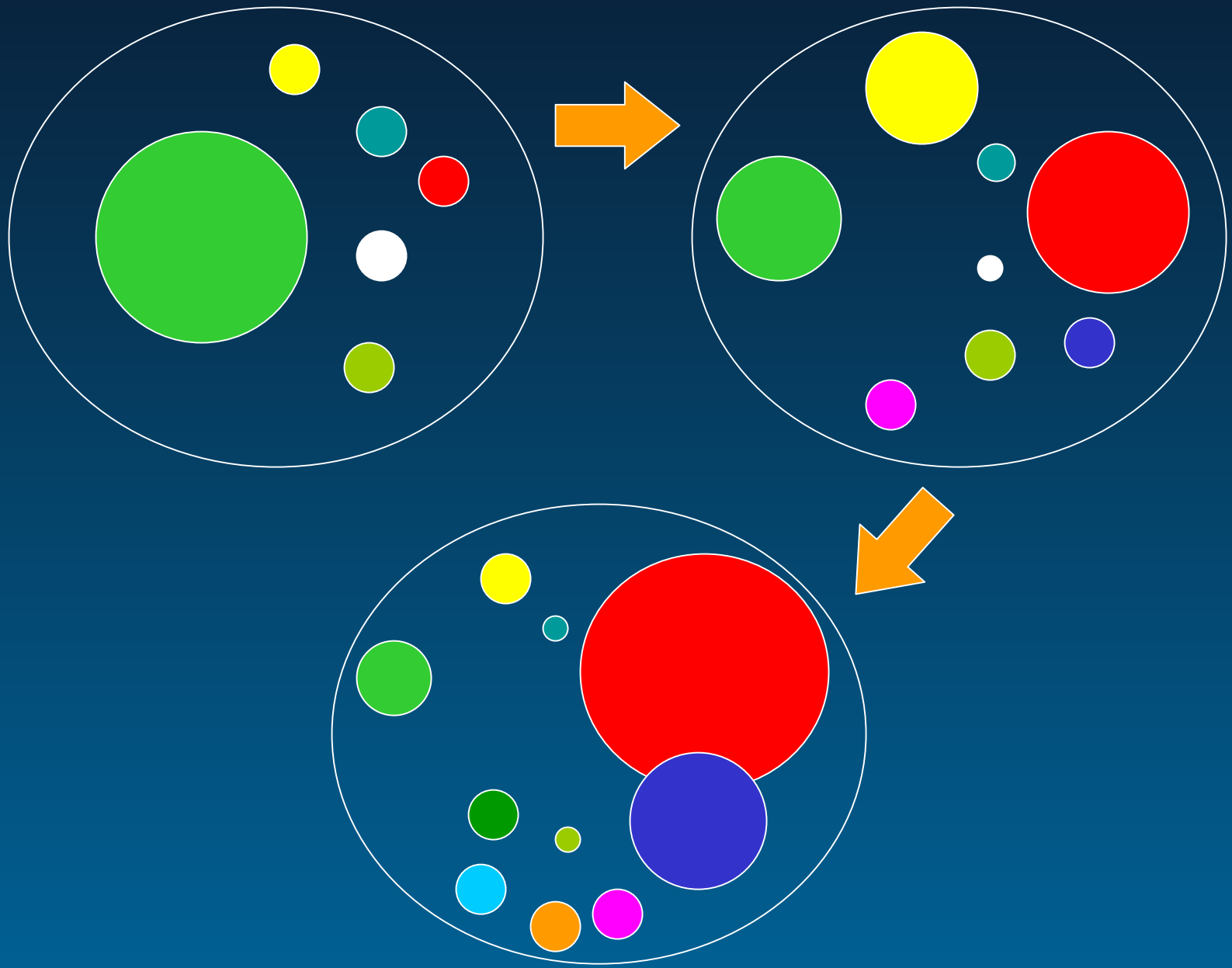


Immune attack

Drug

Repression of the replication


Immune attack
Drug
Repression of the replication



HBV mutations

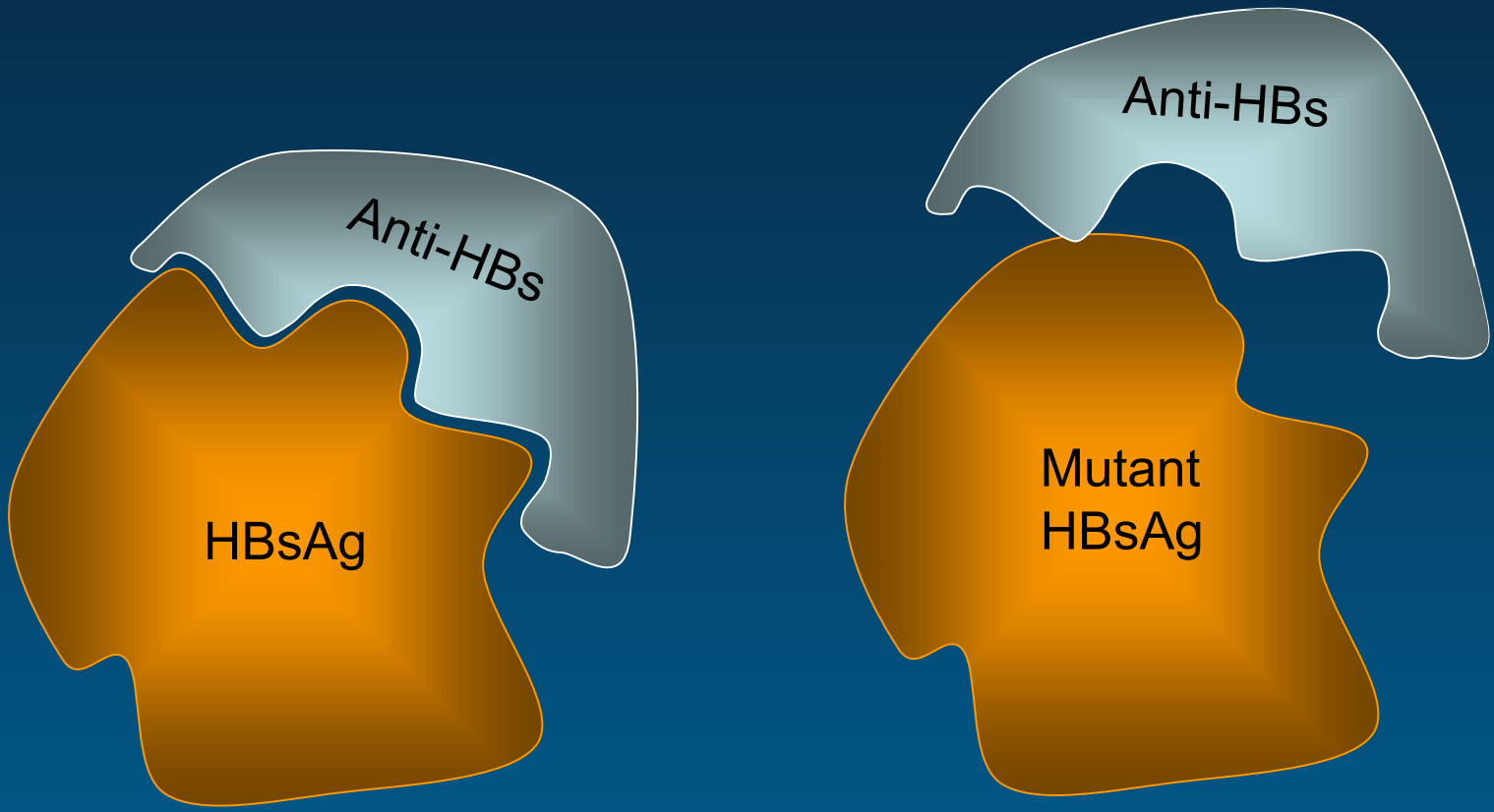
- Various mutations have been described in almost every part of the HBV genome.
- Common features of the mutations:
  - The frequency of the mutations increases with the progression of the infection.
  - They are selected during the time of environmental pressure.
  - They make acceptable changes for both overlapping ORFs.

HBV mutations

- Most studied mutations
  - Immune-, vaccine escape mutations of HBsAg,
  - Precore, core promoter mutations which prevent HBeAg secretion.
  - Mutations leading lamivudine resistance.
  - Mutations associated with HCC.

Vaccine escape mutations

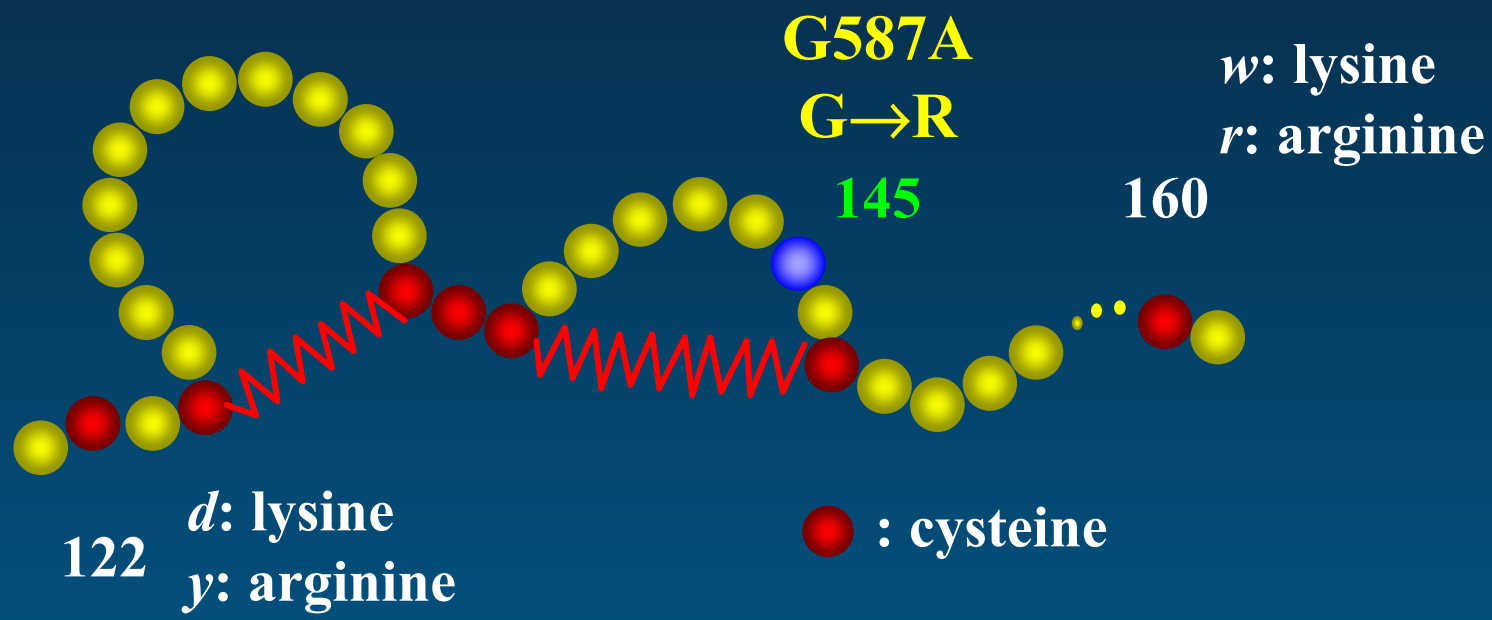
- Vaccines against HBV have proven to be effective in protecting people.
- There have been reports of children who were infected after having completed the full vaccination course.
- Some of these instances are attributed to certain HBV strains carrying mutations on the antigenic “a” determinant of HBsAg


Anti-HBs
HBsAg
Anti-HBs
Mutant
HBsAg

Vaccine escape mutations

- Small surface protein is a hydrophobic protein and has four transmembrane helices, with a hydrophilic two-looped structure spanning from amino acids 124 to 147.*
- This area contains a series of conformational binding sites, including the major antibody determinants of the envelope protein and the primary target of the neutralizing antibody.
- Immune escape mutants are usually clustered in this region.

a determinant and vaccine escape mutants



Missense mutations have been described in codons 126, 129, 133, 141, 144

"a" determinant mutations

- Several studies demonstrated that "*a*" determinant mutations can bind anti-HBs weakly.

Characterization of the Reactivity Pattern of Murine Monoclonal Antibodies Against Wild-Type Hepatitis B Surface Antigen to G145R and Other Naturally Occurring "a" Loop Escape Mutations

MICHEL P. COOREMAN,[1] MARK H. VAN ROOSMALEN,[2] RENÉ TE MORSCHE,[3] CÉCILE M. G. SÜNNEN,[2] ESTHER M. E. SCHOONDERMARK-VAN DE VEN,[3] JAN B. M. J. JANSEN,[3] GUIDO N. J. TYTGAT,[1] PAULINE L. M. DE WIT,[2] AND WILMA P. PAULIJ[2]

J Gastroenterol 2003; 38:244–253

Journal of Gastroenterology

Molecular analysis of antigenicity and immunogenicity of a vaccine-induced escape mutant of hepatitis B virus

TORU SHIZUMA, KIYOSHI HASEGAWA, KAYO ISHIKAWA, TAKUMA NARITOMI, AIKO IIZUKA, NAOKO KANAI, MIHO OGAWA, NOBUYUKI TORII, RIHO JOH, and NAOAKI HAYASHI

Institute of Gastroenterology, Tokyo Women's Medical University, 8-1 Kawadacho, Shinjuku-ku, Tokyo 162-8666, Japan

Escape mutants

Surface gene mutations have been detected in

- Infants born to HBeAg-positive mothers who developed breakthrough infections despite having undergone full passive–active immunoprophylaxis,
- Transplant recipients during the HBIG prophylaxis to prevent infection of liver homograft in immunosuppressed recipients,
- HBsAg negative chronic HBV infections,
- Patients with HCC,
- Asymptomatic HBV carriers.

Questions related to HBsAg mutants

- Do vaccine esacape mutants cause persistent infection?
- Are they harmful to the host?
- Do they break the postexposure prophylaxis?
- Do they infect the people horizontally?
- Can they infect vaccinated people?
- Are there mutations outside *a* determinant which reduce binding of vaccine-induced neutrilizing antibodies?
- Whether the mutation frequency is the same in all genotypes or serotypes?
- Do they decrease the sensitivity of EIAs?
- Are they going to be a public health problem?
- What should be done to prevent the spread of vaccine escape mutations?

HBV vaccination monitoring programme in Singapore

- Active and passive immunoprophylaxis were performed to infants born to HBV carrier mothers.
- Of 345 infants born to HBeAg positive mothers, 41 (12%) were found to be HBV DNA (+)
- None of the 670 infants born to HBeAg negative mothers was HBV DNA (+).
- None of the 107 infants born to HBsAg negative mothers was HBV DNA (+)
- 16 of HBV DNA positive children showed detectable anti-HBs.
- Sequence analysis of HBV DNA identified various "a" determinant mutations.

Table 1 Serological profile of vaccine-escape HBsAg mutants in Singapore

Child	Age (years)	HBsAg	Anti-HBs	Anti-HBc	HBVDNA	'a' epitope mutation
1	13	+	2.0	+	–*	(wild type, Gly_{145}-to-Arg_{145})
2	13	+	–	+	–*	(wild type, Gly_{145}-to-Arg_{145})
3	13	–	7.5	+	–*	(wild type, Pro_{142}-to-Ser_{142}, Gly_{145}-to-Arg_{145})
4	13	–	–	+	–*	(Gly_{145}-to-Arg_{145})
5	13	+	2.5	+	2.3	Gly_{145}-to-Arg_{145}
6	12	+	1.0	+	–*	Phe_{134}-to-Ser_{134}, Gly_{145}-to-Arg_{145}
7	8	+	–	+	3.1	wild type, Gly_{145}-to-Arg_{145}
8	12	+	–	+	11.3	wild type, Lys_{141}-to-Arg_{141}, Gly_{145}-to-Arg_{145}
9	12	–	5.5	+	–*	(Gly_{145}-to-Arg_{145})
10	13	+	–	+	1.5	wild type, Asp_{144}-to-Ala_{144}, Gly_{145}-to-Arg_{145}
11	12	+	–	+	–*	(Gly_{145}-to-Arg_{145})
12	12	+	–	+	25.4	wild type, Thr_{116}-to-Asn_{116} Gly_{145}-to-Arg_{145}
13	13	+	20	+	–*	(Asp_{144}-to-Ala_{144})
14	13	+	3.5	+	63.7	Met_{133}-to-Leu_{133}
15	12	+	–	+	59.7	Gln_{129}-to-His_{129}
16	12	+	–	+	3.3	wild type, Pro_{120}-to-Ser_{120}, Thr_{126}-to-Ala_{126}

*HBVDNA negative by Abbott Genostics assay but positive by PCR.

Mutations were clustered in the *a* determinant of surface antigen.

Summary of the results of Singapore study

- Mutations persisted over at least 13 years.
- HBsAg and anti-HBs coexisted in the children.
- Mutants and wild type coexisted as well.
- During the course of the infection, HBV DNA reduced under the detection limit of hybridization assay in most of the children, yet they were PCR positive.
- HBV DNA tended to remain higher in the patients having HBsAg mutants with altered first loop of the *a* determinant comparing to those with an altered second loop.
- Mutants were detected in some of the mothers.

Vaccine escape mutants in postexposure prophylaxis of newborns

- Almost 4% of babies born to HBsAg positive mothers are infected with HBV despite active and passive immunization.
- If mother has a replicative infection, more than 10% are infected.
- HBsAg *a* determinant mutants can be detected in 25-50% of these infections.
- Mutants can be detected for more than 15 years in the bloodstream.
- Mutation rate in other parts of HBsAg is close to those found in elsewhere in HBV genome.
- Mostly mothers of infected babies has no demonstrable HBsAg mutants.
- Some of the babies have chronic hepatitis in their follow-up.

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Unanswered questions

- How do the mutations evolve?
- Some mutants may bind anti-HBs, then how do they persist?
- If the only mechanism is the selection under the immune pressure, why does the wild type persist together with mutants?

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Journal of Infection (2000) **41**, 260–264
doi:10.1053/jinf.2000.0751, available online at http://www.idealibrary.com on IDEAL®

Intra-familial Evidence of Horizontal Transmission of Hepatitis B Virus Surface Antigen Mutant G145R

Chong Jin Oon[1], Wei Ning Chen[*2], Kian Sim Goo[1] and Kee Tai Goh[3]

Table I. Intra-familial detection and possible horizontal transmission of G145R HBsAg mutant.

	Sampling	HBsAg/ anti-HBs	HBV DNA	HBeAG/ anti-HBe	ALT	'a' Determinant
Family 2						
Mother	1985	+/−	49.8	+/+	n.d.	WT
	1990	+/−	−	−/+	n.d.	WT
	1997	+/4.0	−	n.d./+	14	WT
Father	1991	−/410.0	−	n.d./+	n.d.	WT
	1993	−/300.0	−	n.d./+	17	WT
	1995	−/450.0	−	n.d./+	15	WT/Gly145Arg
Child 1	1985 (week 12)	−/14.2	−	−/+	n.d.	−
	1985 (month 6)	+/−	n.d.	n.d./+	n.d.	WT/Gly145Arg
	1995	+/−	6.0	n.d./+	18	WT/Gly145Arg
	1996	+/1.5	12.0	n.d./+	**251**	WT/Gly145Arg
Child 2	1991	−/60.0	−	n.d./−	n.d.	−
	1995	−/5.0	−	n.d./−	13	−
	1997	−/4.5	−	n.d./−	11	−
Family 3						
Mother	1985	+/n.d.	92.0	n.d./+	n.d.	WT
	1995	+/4.0	52.1	n.d./+	20	WT
	1998	+/n.d.	74.3	n.d./+	**105**	WT
Father	1995	−/4750.0	−	n.d./+	22	WT/Gly145Arg
	1997	−/3850.0	−	n.d./+	28	−
Child 1	1985 (day 1)	−/−	n.d.	n.d./n.d.	n.d.	−
	1986	+/−	n.d.	n.d./+	n.d.	Gly145Arg
	1995	+/−	3.5	n.d./+	13	Gly145Arg
	1997	+/−	11.3	n.d./+	12	Gly145Arg
	1998	+/−	6.5	n.d./+	30	Gly145Arg
Child 2	1995	−/16.0	−	n.d./−	15	−
	1998	−/10.5	−	n.d./−	**45**	−
Child 3	1995	−/420.0	−	n.d./−	13	−
	1998	−/340.0	−	n.d./−	15	−

n.d. (not determined); + (positive); − (negative); anti-HBs (in IU/ml); HBV DNA (in pg/ml); ALT (alanine aminotransferase, in IU/l); WT (Wild Type HBV).

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Journal of Gastroenterology and Hepatology (2001) **16**, 1373–1377

ESCAPE MUTANTS OF HEPATITIS B VIRUS

Prevalence of vaccine-induced escape mutants of hepatitis B virus in the adult population in China: A prospective study in 176 restaurant employees

CHUAN HE,* FUMIO NOMURA,† SAKAE ITOGA,† KAZUMASA ISOBE* AND TOSHIAKI NAKAI*

	Before vaccination			After vaccination		
	n	HBsAg (+)	HBV DNA (+)	n	HBsAg (+)	HBV DNA (+)
Anti-HBs (+)	0	0	0	167 (94.9)	1	4 (2.3)
Anti-HBs (-)	176	0	0	9 (5.1)	1	2
Total	176	0	0	176	2 (1.1)	6 (3.4)

Gly 145 Ala

Thr 126 Ser

Thr 126 Asn

Ile 126 Asn

Licensed Recombinant Hepatitis B Vaccines Protect Chimpanzees Against Infection With the Prototype Surface Gene Mutant of Hepatitis B Virus

Norio Ogata,[1,2] Paul J. Cote,[3] Alessandro R. Zanetti,[4] Roger H. Miller,[1,5] Max Shapiro,[6] John Gerin,[3] and Robert H. Purcell[1]

(HEPATOLOGY 1999;30:779-786.)

- 4 chimps were adminestered regular recombinant vaccines.
- 2 were not vaccinated.
- None of the vaccinees can be infected with AS serum (mutant + wild type virus).
- Unvaccinated chimps were infected with both mutant and wild type.

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Mutations in T helper cell epitopes may diminish the anti-HBs response against HBV

Variants of Two Major T Cell Epitopes Within the Hepatitis B Surface Antigen Are Not Recognized by Specific T Helper Cells of Vaccinated Individuals

Tanja Bauer, Klaus Weinberger, and Wolfgang Jilg

Table 6. Frequency of INF-γ–Secreting Cells in Response to Epitope P4 wt and Its Naturally Occurring Variants

	Number of INF-γ–Secreting Cells/10^6 PBMCs									
Antigen	Donor 2	Donor 7	Donor 10	Donor 13	Donor 16	Donor 17	Donor 19	Donor 26	Donor 27	Donor 30
P4 wt	38 ± 3	57 ± 4	39 ± 3	49 ± 3	51 ± 3	51 ± 5	57 ± 2	49 ± 4	39 ± 1	33 ± 2
P4/M1	**3 ± 0**	**5 ± 1**	**4 ± 0**	48 ± 2	49 ± 2	47 ± 2	50 ± 2	**4 ± 2**	37 ± 3	31 ± 2
P4/M2	35 ± 2	49 ± 3	40 ± 2	40 ± 3	48 ± 5	40 ± 2	50 ± 4	55 ± 4	40 ± 2	37 ± 2
P4/M3	33 ± 3	46 ± 4	37 ± 3	46 ± 2	44 ± 2	41 ± 4	47 ± 4	49 ± 3	33 ± 3	39 ± 4
P4/M4	34 ± 3	40 ± 5	38 ± 4	39 ± 3	39 ± 3	37 ± 2	34 ± 5	35 ± 4	31 ± 2	30 ± 3
P4/M5	**3 ± 1**	**3 ± 2**	**2 ± 1**	42 ± 3	57 ± 2	48 ± 4	36 ± 2	**5 ± 1**	37 ± 1	29 ± 2
P4/M6	**4 ± 1**	**6 ± 1**	**3 ± 1**	48 ± 3	50 ± 3	43 ± 2	43 ± 1	**2 ± 2**	39 ± 2	34 ± 3
P4/M7	30 ± 2	48 ± 4	35 ± 2	46 ± 2	49 ± 4	42 ± 2	44 ± 3	46 ± 2	35 ± 4	38 ± 2
P4/M8	31 ± 2	46 ± 2	39 ± 3	47 ± 5	44 ± 2	44 ± 4	39 ± 2	42 ± 3	36 ± 3	35 ± 2
P4/M9	32 ± 3	49 ± 3	30 ± 2	43 ± 3	46 ± 5	39 ± 2	48 ± 3	46 ± 4	48 ± 5	28 ± 3
P4/M10	37 ± 2	53 ± 2	34 ± 2	44 ± 2	47 ± 3	47 ± 3	42 ± 5	47 ± 3	46 ± 2	36 ± 2
P4/M11	36 ± 2	55 ± 2	35 ± 2	42 ± 2	40 ± 2	37 ± 2	40 ± 3	50 ± 2	37 ± 3	37 ± 3
HIV	6 ± 2	7 ± 2	5 ± 2	9 ± 2	8 ± 2	5 ± 2	6 ± 1	9 ± 2	5 ± 0	8 ± 2

NOTE. PBMCs were tested for cytokine secretion upon stimulation with either wild-type epitope (P4 wt) or the P4-derived variants (P4/M1 to P4/M11). An HIV-env peptide was used as negative control (HIV). Numbers shown are the numbers of spots per 10^6 PBMCs (mean ± SD) calculated as described in Materials and Methods. Significantly changed responses compared with the corresponding wild-type peptide P4 wt are bold and underlined.

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ELSEVIER

Vaccine 20 (2002) 639–640

Vaccine

www.elsevier.com/locate/vaccine

Letter to the Editor

Frequent occurrence of hepatitis B virus surface antigen mutants in subtype *adw* in vaccinated Singapore infants

	Prevalence in HBV carriers	Prevalence in vaccinated infants
adw	%65.7	%96.2
adr	%30.8	%3.8

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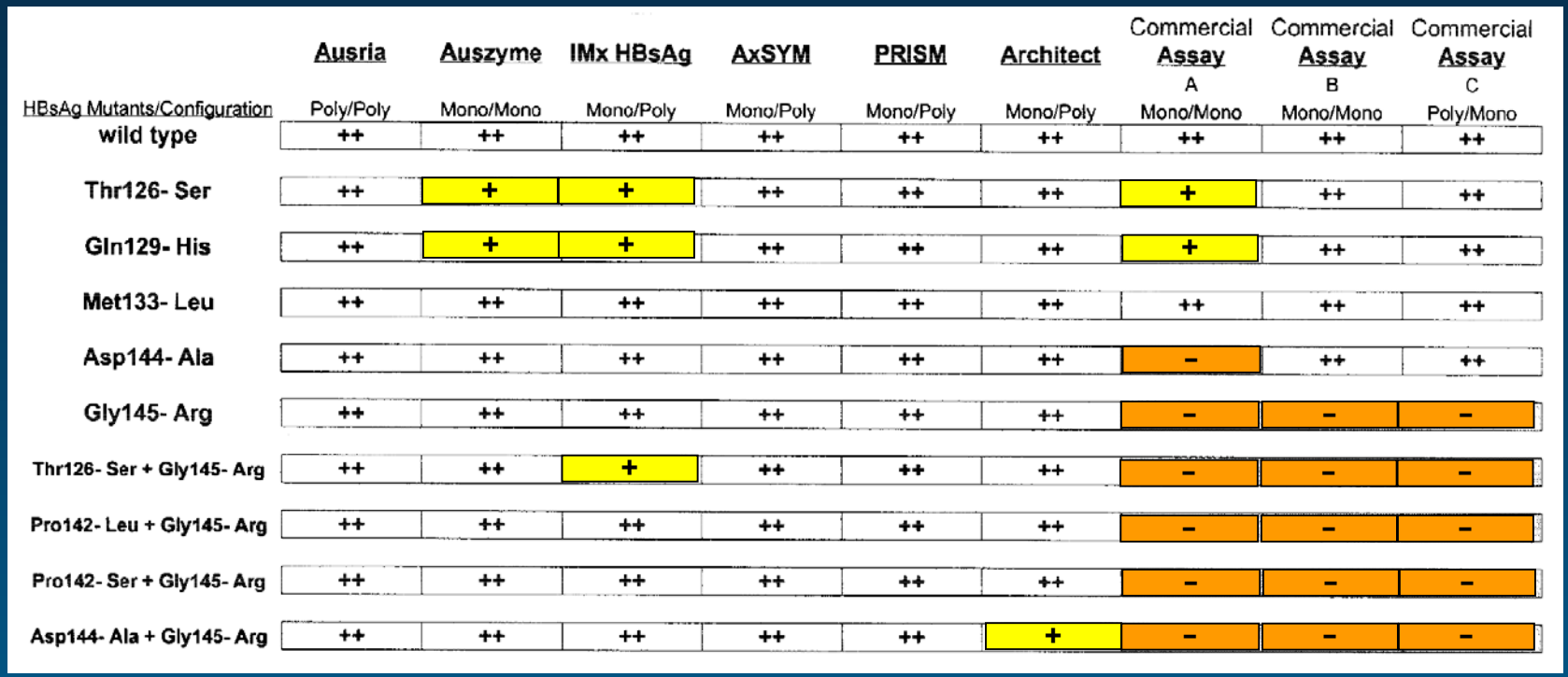
Detection of HBsAg mutants by EIA

- Antibodies used in EIA may not detect the HBsAg mutants.
- If they use monoclonal antigens, this is more likely to be a problem.

Journal of Medical Virology 59:19–24 (1999)

Immunoassay Detection of Hepatitis B Surface Antigen Mutants

Paul F. Coleman, Y.-C. Jack Chen, and Isa K. Mushahwar*
Viral Discovery Group, Abbott Diagnostics Division, Abbott Laboratories, North Chicago, Illinois


Ausria
Auszyme
IMx HBsAg
AxSYM
PRISM
Architect
Commercial Assay A
Commercial Assay B
Commercial Assay C
HBsAg Mutants/Configuration
Poly/Poly
Mono/Mono
Mono/Poly
Mono/Poly
Mono/Poly
Mono/Poly
Mono/Mono
Mono/Mono
Poly/Mono
wild type
++ ++ ++ ++ ++ ++ ++ ++ ++
Thr126- Ser
++ + + ++ ++ ++ + ++ ++
Gln129- His
++ + + ++ ++ ++ + ++ ++
Met133- Leu
++ ++ ++ ++ ++ ++ ++ ++ ++
Asp144- Ala
++ ++ ++ ++ ++ ++ – ++ ++
Gly145- Arg
++ ++ ++ ++ ++ ++ – – –
Thr126- Ser + Gly145- Arg
++ ++ + ++ ++ ++ – – –
Pro142- Leu + Gly145- Arg
++ ++ ++ ++ ++ ++ – – –
Pro142- Ser + Gly145- Arg
++ ++ ++ ++ ++ ++ – – –
Asp144- Ala + Gly145- Arg
++ ++ ++ ++ ++ + – – –

HBsAg Mutants/Configuration	Ausria	Auszyme	IMx HBsAg	AxSYM	PRISM	Architect	Commercial Assay A	Commercial Assay B	Commercial Assay C
	Poly/Poly	Mono/Mono	Mono/Poly	Mono/Poly	Mono/Poly	Mono/Poly	Mono/Mono	Mono/Mono	Poly/Mono
wild type	++	++	++	++	++	++	++	++	++
Asn40- Ser	++	++	++	++	++	++	++	++	++
Pro111- Thr	++	++	+	++	++	++	++	++	++
Thr Thr115,116- Ile Ile	++	++	++	++	++	++	++	+	++
Thr118- Ser	++	++	++	++	++	++	++	++	++
Pro120- Gln	++	-	++	++	++	++	-	++	++
Serum	++	-	++				-		
Thr131- Ile	++	++	++	++	++	++	++	++	++
Pro135- Ser	++	++	++	++	++	++	-	-	++
Lys141- Glu	++	-	++	++	++	++	-	-	++
Pro142- Leu	++	++	++	++	++	++	-	-	++
Pro142- Ser	++	++	++	++	++	++	++	-	++
Gly145- Ala	++	++	++	++	++	++	+	++	++
Gly145- Lys	++	++	++	++	++	++	-	-	-
Thr148- His	++	+	++	++	++	++	-	++	++
Ser154- Trp	++	+	+	++	++	++	-	-	-
MetMetMet196-198- SerSerSer	++	++	++	++	++	++	++	++	++
Clinical seqs.									
Insertion seq. A (adw2)	++	-	-	-	-	+	-	-	++
Insertion seq. B	++	-	-	-	-	-	-	-	-
Serum (ayw1)	++	-	-				-		

Chen WN and Oon CJ. J Clin Microbiol, 2000

- 63 adult, 15 children vaccinee who were HBsAg negative and anti-HBe positive were studied
- 8/63 (13%) adult HBV DNA(+)
- 3/15 (20%) children HBV DNA(+)
- All patients had mutations in major hydrophilic region of HBsAg

- ~~Do vaccine esacape mutants cause persistent infection?~~
- ~~Are they harmful to the host?~~
- ~~Do they disturb the postexposure prophylaxis?~~
- ~~Do they infect the people horizontally?~~
- ~~Can they infect vaccinated people?~~
- ~~Are there mutations outside a determinant which reduce binding of vaccine-induced neutrilizing antibodies?~~
- ~~Whether the mutation frequency is the same in all genotypes or serotypes?~~
- ~~Do they decrease the sensitivity of EIAs?~~
- Are they going to be a public health problem?
- What should be done to prevent the spread of vaccine escape mutations?

Changes of Hepatitis B Surface Antigen Variants in Carrier Children Before and After Universal Vaccination in Taiwan

HONG-YUAN HSU,[1,2] MEI-HWEI CHANG,[2] SHWU-HUEY LIAW,[3] YEN-HSUAN NI,[2] AND HUEY-LING CHEN[2]

1984	8/103	7.8%
1989	10/51	19.6%
1994	9/32	28.1%
Vaccinated p.	12/33	36.4%
Nonvaccinated	15/153	9.8%

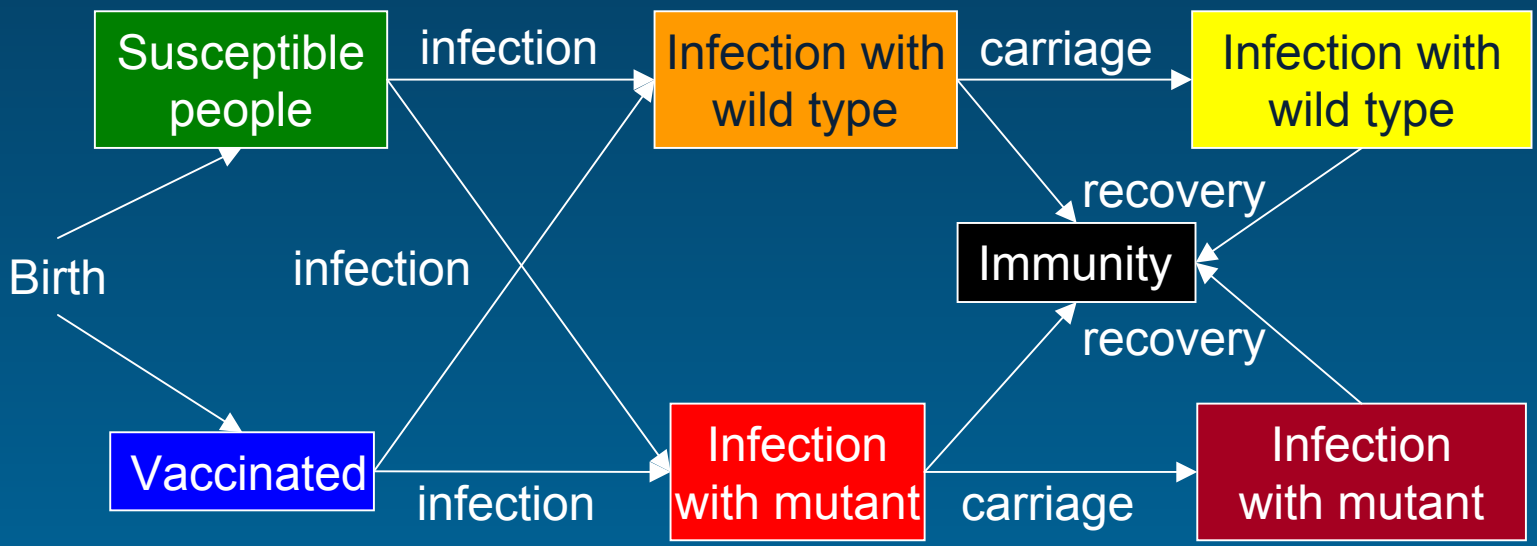
140-149 mutants more prevalent in vaccinees.

Vaccine-escape mutants may become a prodominant strain in the future

Journal of Viral Hepatitis, 1998, **5** (Suppl 2), 25–30

Current status of HBV vaccine escape variants – a mathematical model of their epidemiology

J. N. Wilson[1], D. J. Nokes[2] and W. F. Carman[3] [1]*Wellcome Trust Centre for the Epidemiology of Infectious Disease, University of Oxford, Oxford OX1 3PS,* [2]*Department of Biological Sciences, University of Warwick, Coventry,* [3]*Institute of Virology, University of Glasgow, Church Street, Glasgow, UK*



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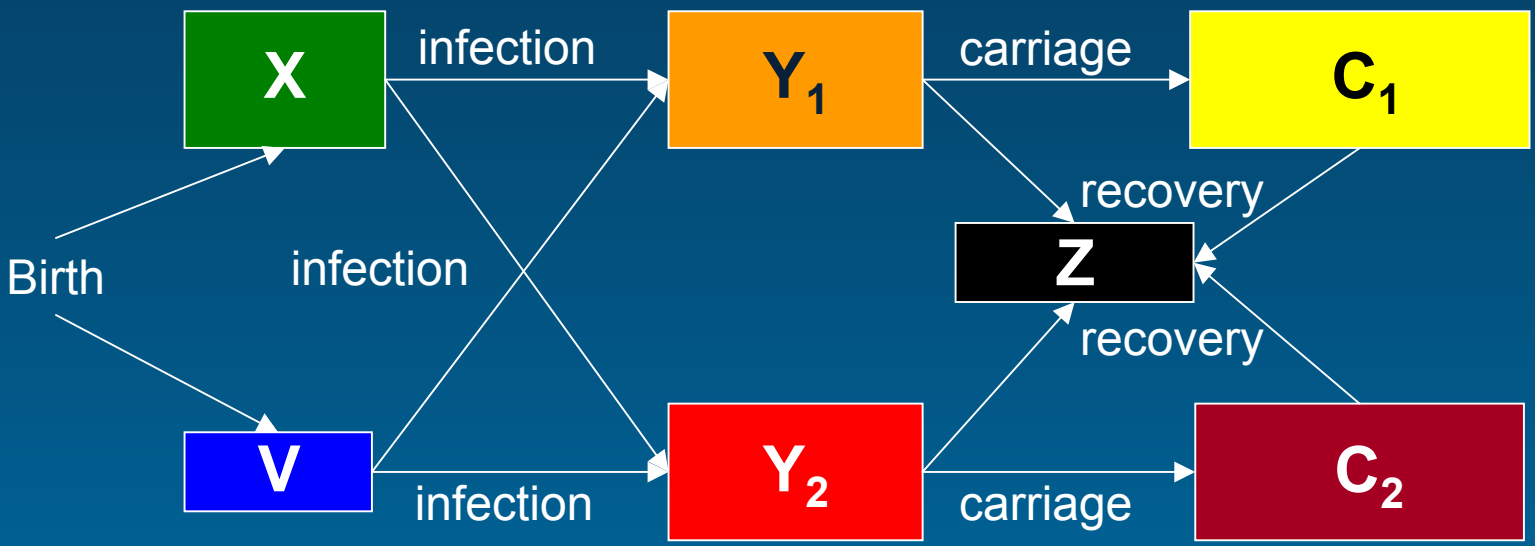
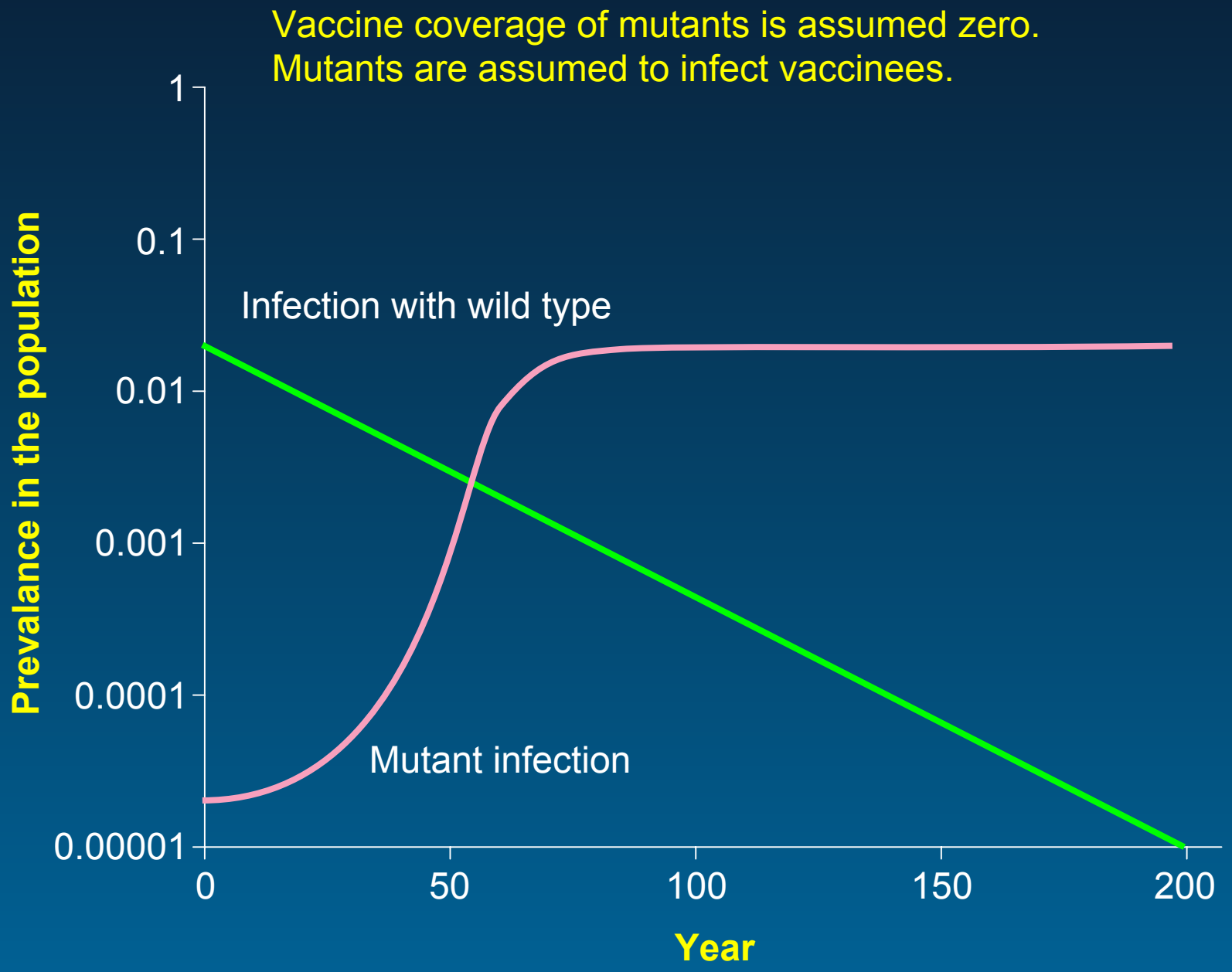


Assumptions

Parameter	Value	Symbol	Ref
Rate of recovery from chronic infection (average 40 years)	1/40 years	σ	[24]
Rate of recovery from primary infection (average $2^{1}/_{2}$ months)	1/0.2 years	γ	[24]
Population birth/death rate (life expectancy 43 years)	1/43 years	μ	[24]
Relative infectiousness of acutes and carriers	16%	η	[24]
Relative infectiousness of variant	99%	α	?
Probability of developing chronic infection	30%	p	[17]
Probability of vertical transmission from acute mother	71%	b	[24]
Basic reproductive number	5	R_0	[17]
Vaccine efficacy	95%	ϕ	[19]
Vaccine coverage	0–100%	ν	
Vaccine cross-protection	0–100%	c	


Vaccine coverage of mutants is assumed zero.
Mutants are assumed to infect vaccinees.
Prevalance in the population
1
0.1
0.01
0.001
0.0001
0.00001
Infection with wild type
Mutant infection
0
50
100
150
200
Year

- ~~Do vaccine esacape mutants cause persistent infection?~~
- ~~Are they harmful to the host?~~
- ~~Do they disturb the postexposure prophylaxis?~~
- ~~Do they infect the people horizontally?~~
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- What should be done to prevent the spread of vaccine escape mutations?

What should be done to prevent the spread of vaccine escape mutations?

- Is it necessary?
- Formulation of vaccines may be changed:
  - PreS1-S2 antigens may be added.
    - ?PreS1-S2 mutations
  - Mutant antigens can be added.

Conclusions

- HBsAg mutants may cause persistent infection.
- May be associated with chronic hepatitis.
- They interfere with post exposure prophylaxis.
- They may prevent the detection of HBsAg in HBV infection.
- They are more likely to be present with some serotypes (adw>adr).
- They can infect the unvaccinated peoples horizontally.
- There has been no clear evidence that they can infect the vaccinated peoples.
- They may be prevalent in the future.

Future studies

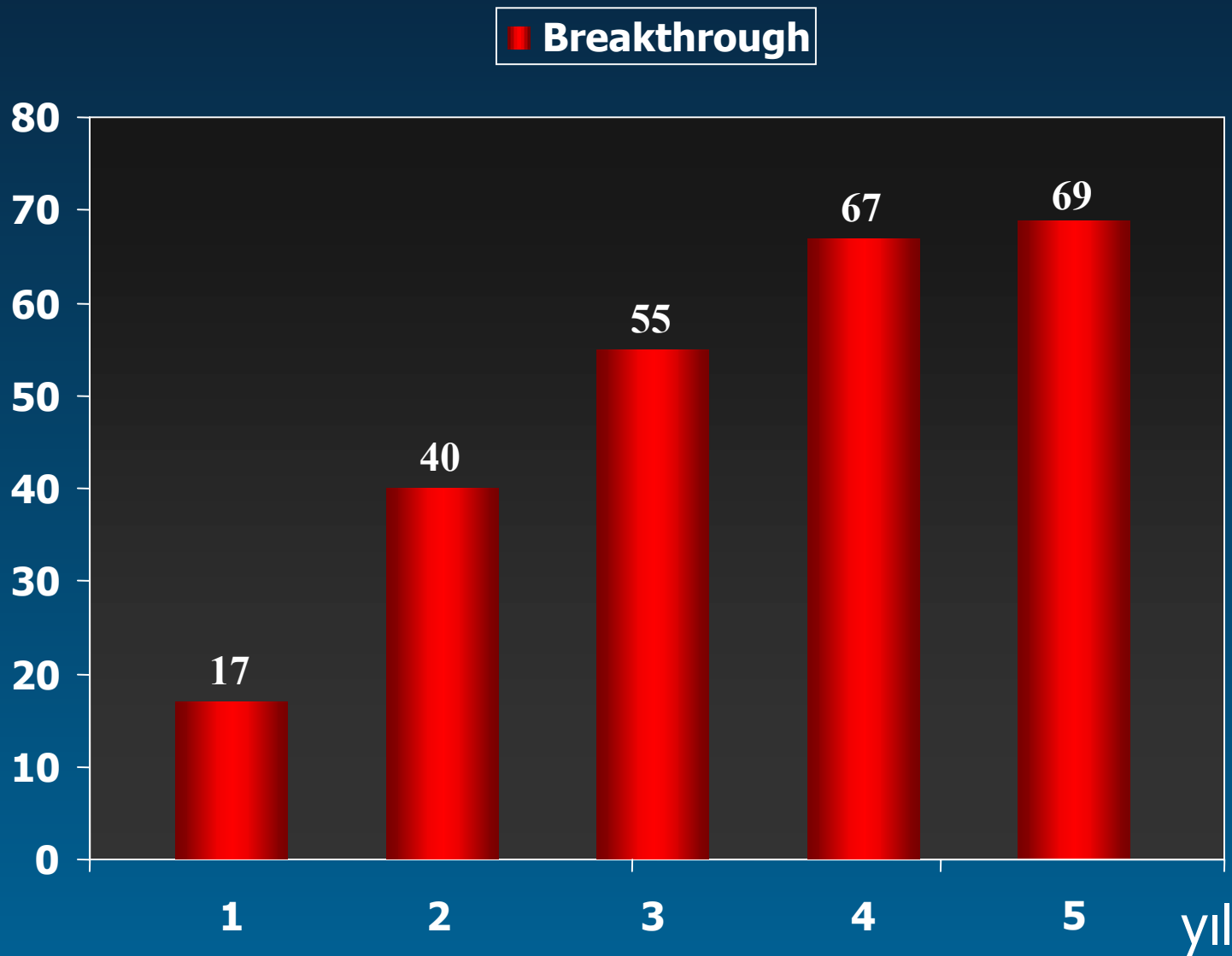
- Easy methods to detect the mutations.
- Epidemiologic prospective and clinical studies are needed
  - To monitor the occurrence and distribution of the mutants
  - To test the risk of transmission in susceptible and immunized people.
  - To detect its pathogenicity.

- Currently, regular vaccines are safe and effective. There is no concincing evidence to add the new antigens to the vaccines in the near future. We need more data coming from well designed studies.

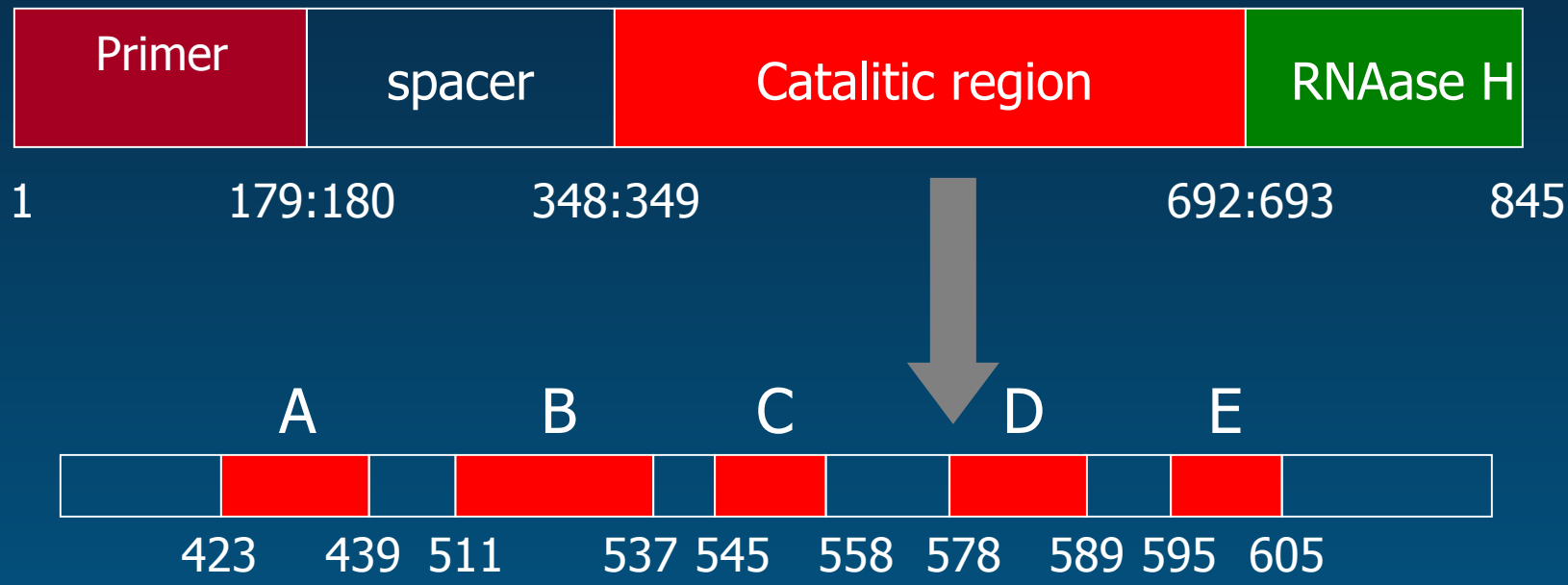
Immunogenicity of lamivudine-resistant mutants

- Lamivudine is a potent inhibitor of RNA-dependent DNA polymerase of HBV.
- It effectively reduces viral burden in chronic HBV infection.
- It reduces ALT levels to normal range
- Improves the liver histology.
- But long-term treatment with lamivudine may, however lead to resistance as the result of the generation of mutations at the YMDD locus in the C domain of the polymerase gene.

Resistance problem for long-term lamivudine use

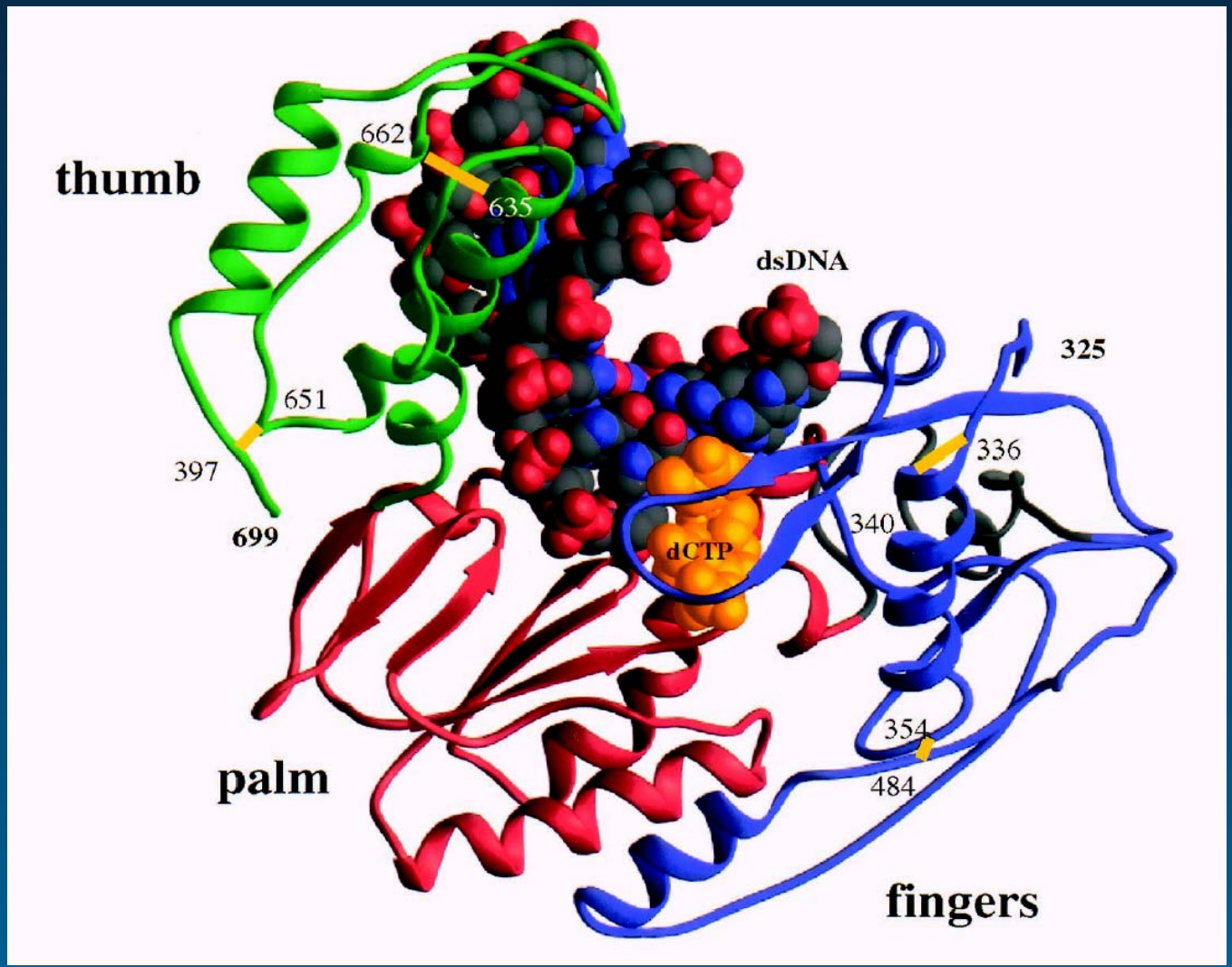


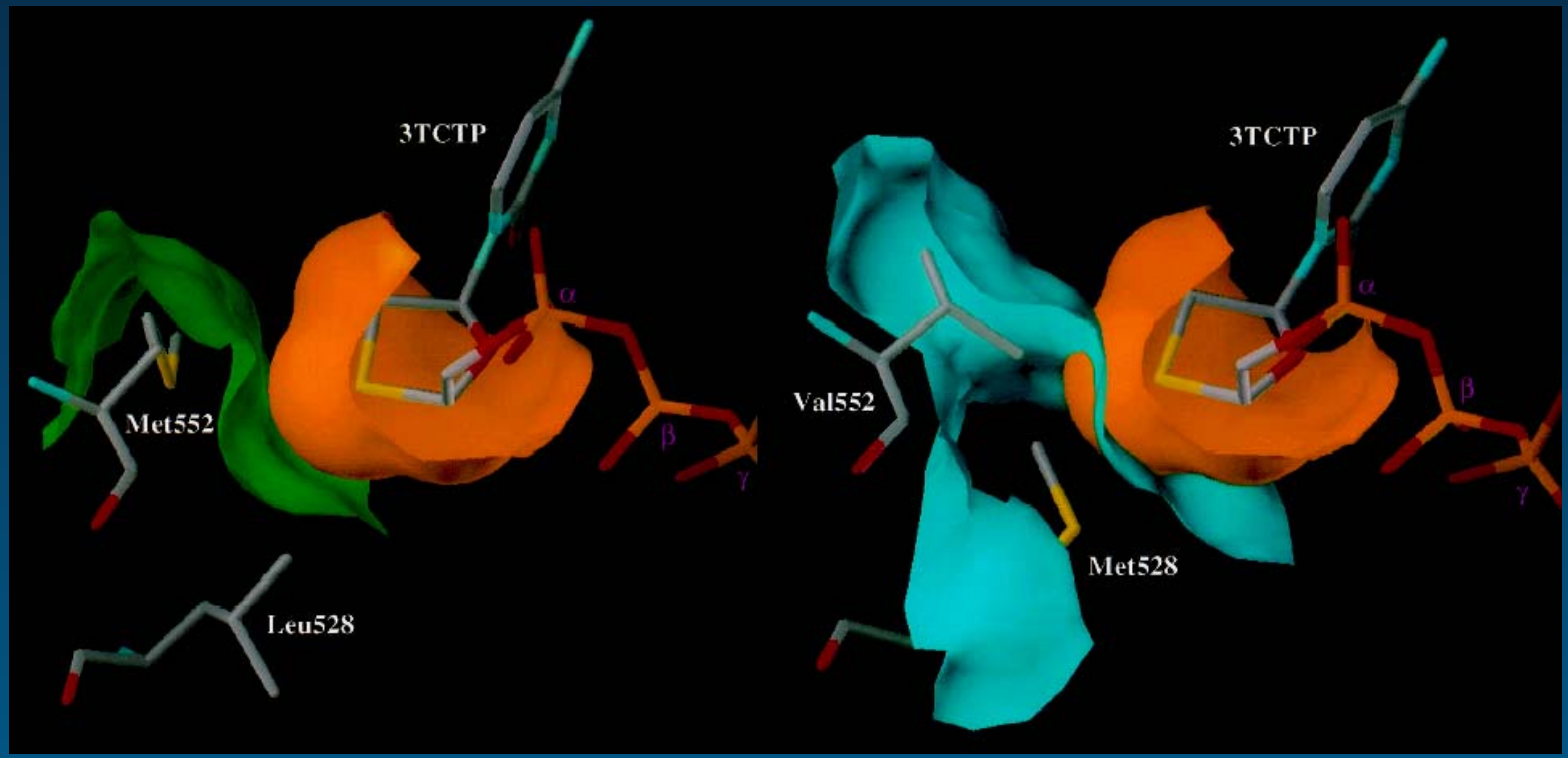
Guan APDW 2001



L528M
M552V/I

HBV	Lamivudine	Clevudine	L(-)Fd4C,	Entecavir	Emtricitabine	L-dC	L-dT	Adefovir
Wild-type	1	1	1	1	1	1	1	1
L180M	3	23	3	1	11	~10	~10	1
M204I	389	>189	N/A	860	>42	>300	>300	4
L180M/M204V	8620	>189	233	180	>42	>300	>300	2

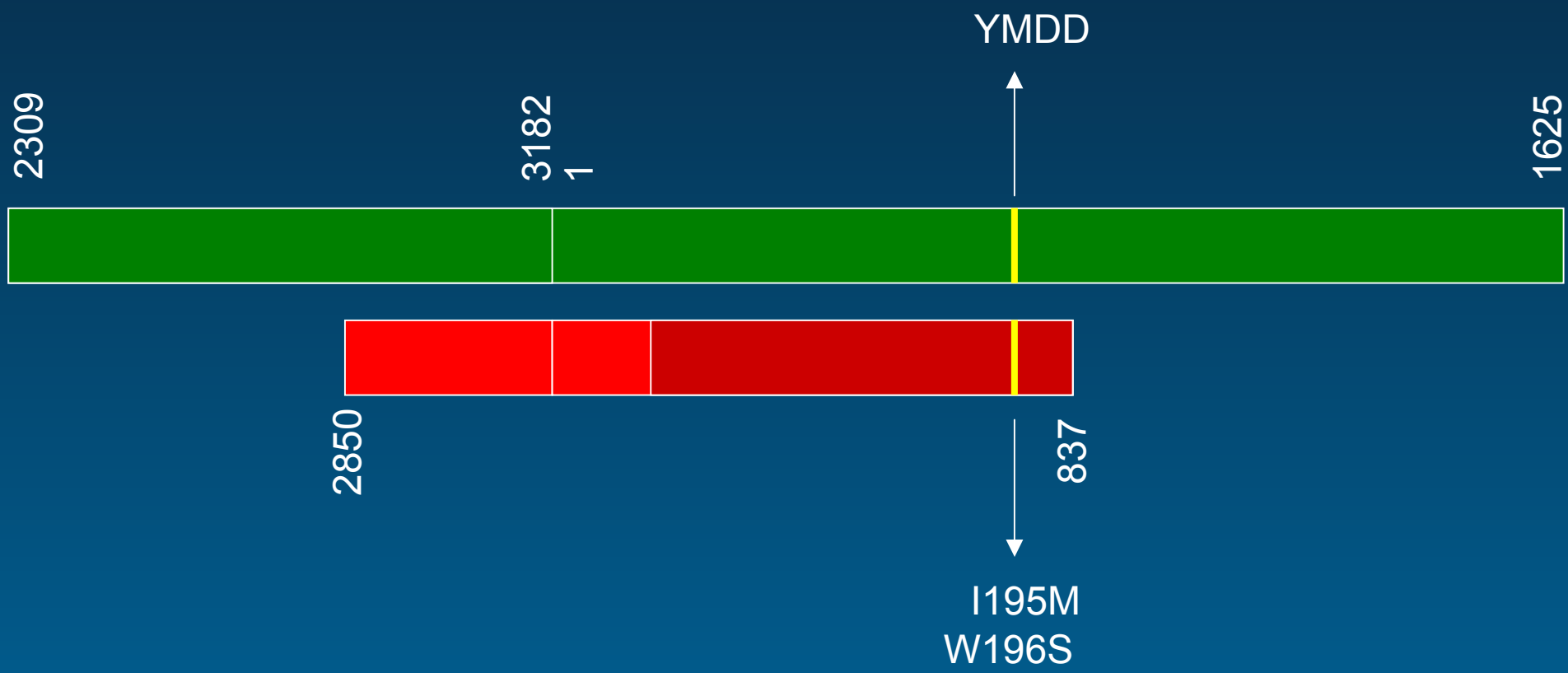

thumb
662
635
dsDNA
325
651
336
397
340
699
dCTP
354
palm
484
fingers

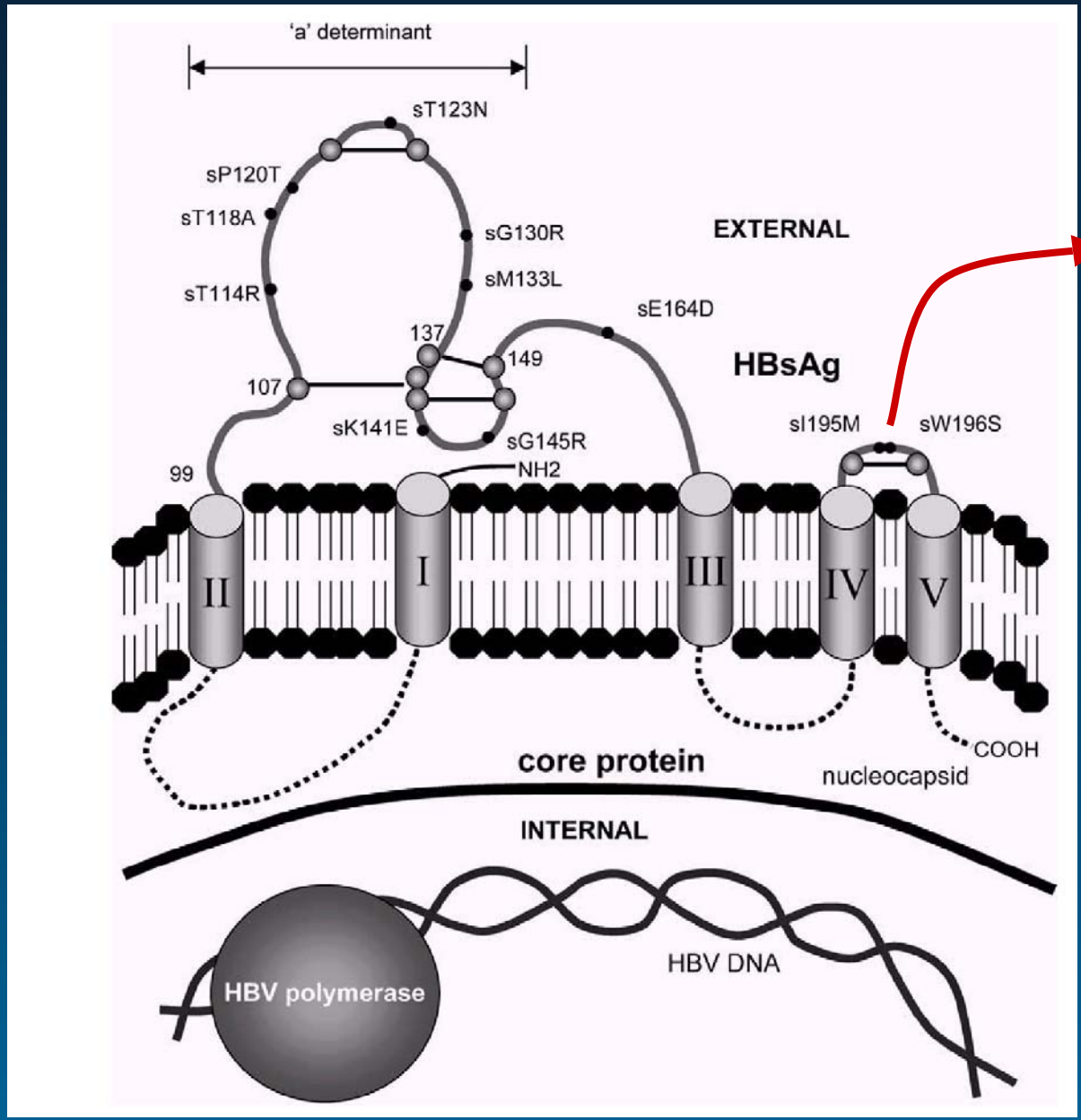

3TCTP
α
β
γ
Met552
Leu528
3TCTP
α
β
γ
Val552
Met528

Major mutations leading LAM resistance:

Group 1:		HBsAg change
M552V (M204V)	C domain	I195M
L528M (L180M)	B domain	No change
Group 2		
M552I (M204I)	C domain	W196S / W196L stop
Other		
S565P		S210R

HBsAg changes in lamivudine resistance



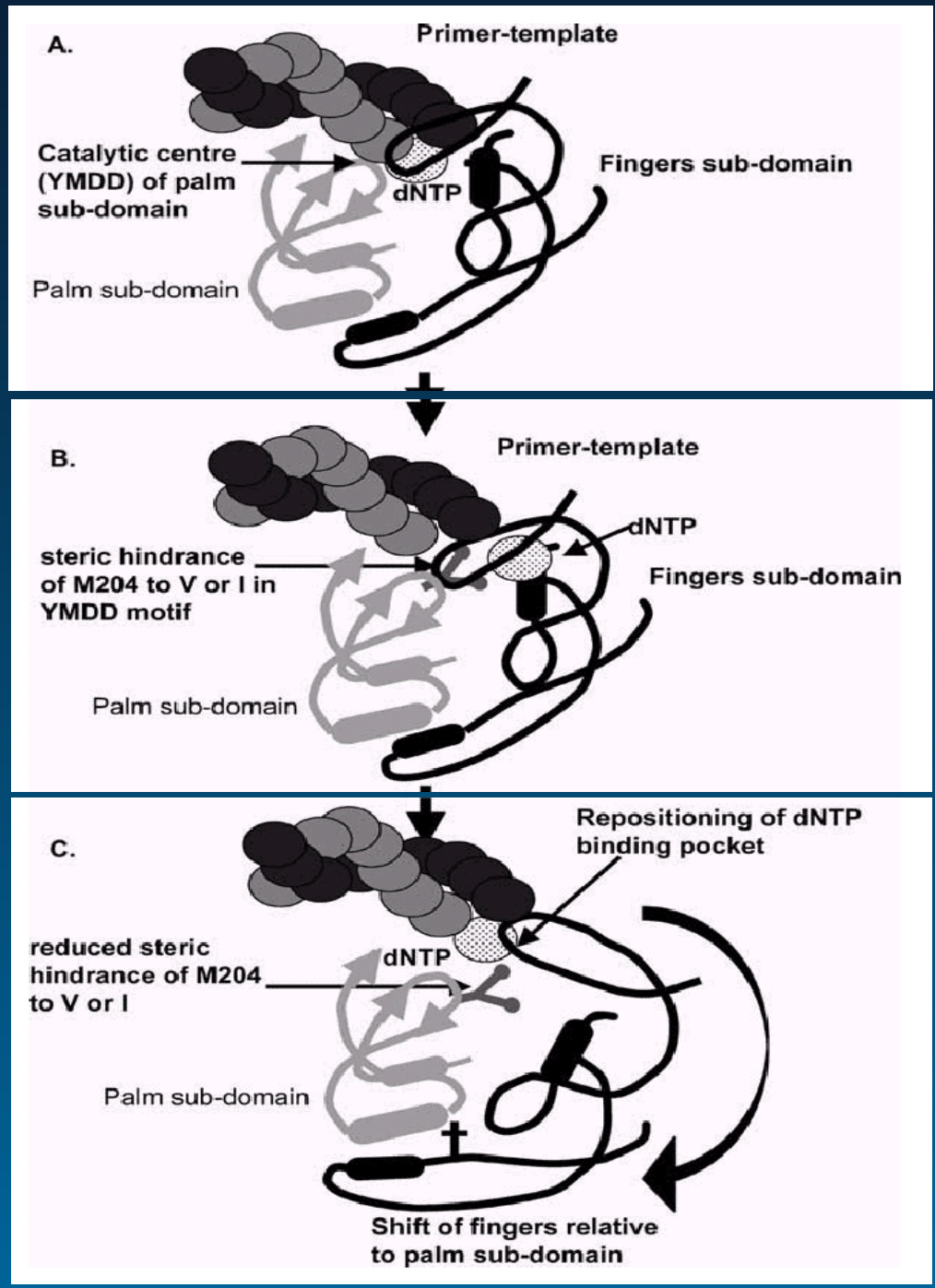


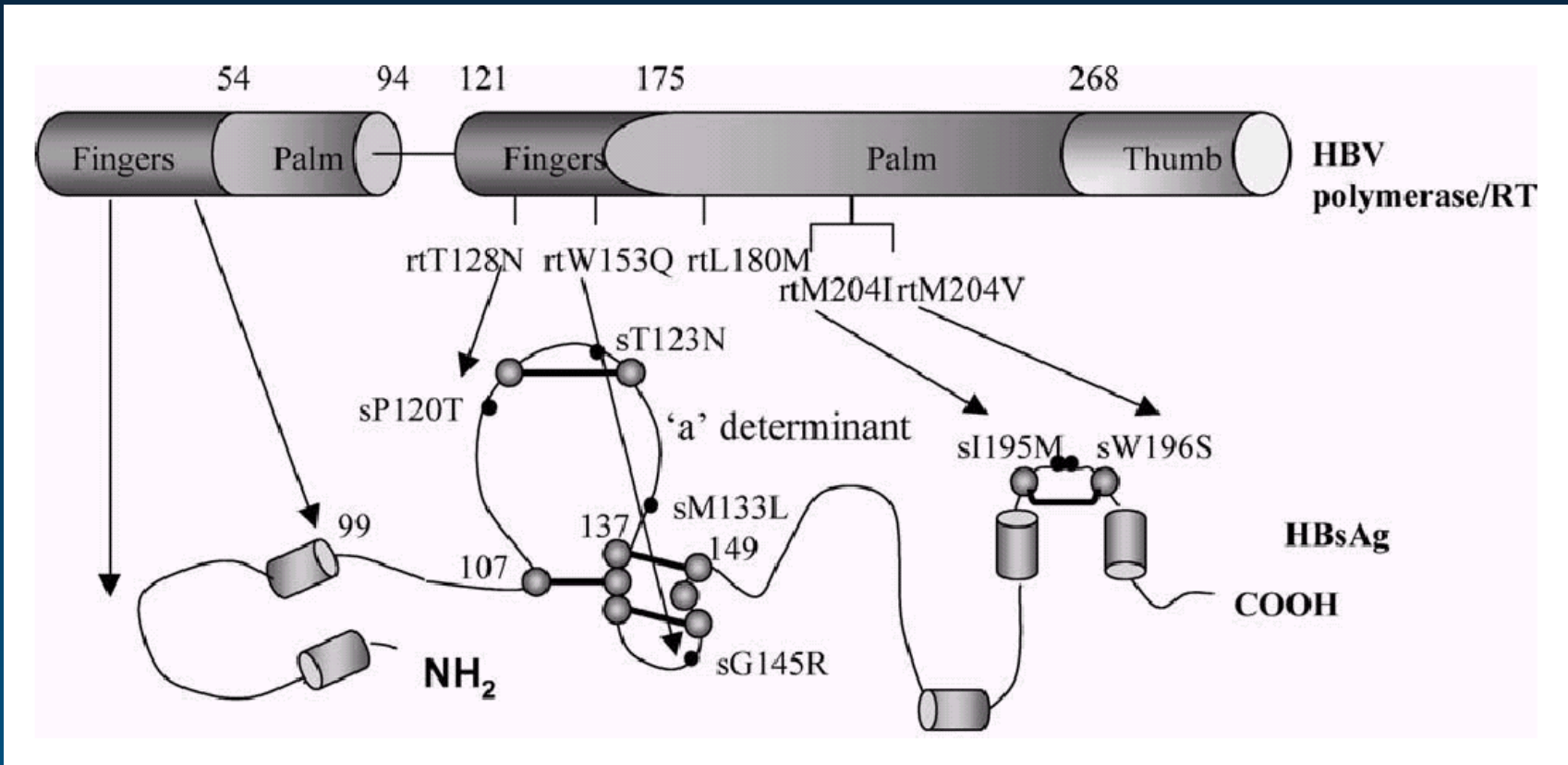
AntiHBs binding capacity of these mutants is very low

There is no data regarding the infectivity of these mutants.

Experimental and epidemiologic data are needed.

- Replication capacity of LAM-resistant mutations decreases.
- Reduced replication is another selective pressure for the virus.
- Compensatory mutations which restore the replication effectiveness in HBV genome may be selected.
- Some mutations in the finger region of the polymerase gene increase the binding of nucleotides to the palm region of the enzyme. This increases the replication capacity.


A.
Primer-template
Catalytic centre (YMDD) of palm sub-domain
dNTP
Fingers sub-domain
Palm sub-domain
B.
Primer-template
dNTP
steric hindrance of M204 to V or I in YMDD motif
Fingers sub-domain
Palm sub-domain
C.
Repositioning of dNTP binding pocket
reduced steric hindrance of M204 to V or I
dNTP
Palm sub-domain
Shift of fingers relative to palm sub-domain



This compensatory mutation changes the a determinant of HBsAg

After extensive antiviral use, mutants may be more prevalent??

Conclusions

- Despite effective vaccination, chronic HBV infection is remaining as a major health problem.
- Virus develops many mechanisms to escape from immune or drug exposure, to increase replicative and maintenance mechanisms.
- But human being is capable to overcome all these mechanisms.
- In the near future, vaccination and effective antivirals can eradicate the virus from the world.